STUDIES ON COMPLEMENT-FIXING ANTIBRAIN ANTIBODIES IN MULTIPLE SCLEROSIS

By
BJÖRN RYBERG

Lund 1981

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Abstract

Employing the complement fixation technique, which appears to discriminate between IgG specifically and non-specifically bound to CNS preparations, the present study delineated 7 specificities of antibrain antibodies (ABA) in multiple sclerosis (MS).

The distribution of these IgG antibodies between serum and CSF indicated that they are produced on both sides of the blood-brain barrier in proportions that show a great interindividual variation.

ABA were demonstrable in serum and/or CSF in 92% of MS patients without disturbing anticomplementary activity. The absence of ABA in most patients with other neurological diseases shows that the mere destruction of CNS tissue is not sufficient for their production.

ABA titres in serum and CSF were correlated with a more malignant course and disability in MS, but did not reflect the individual bouts and remissions. Irrespective of the mechanism of induction of these autoantibodies in MS - an excessive immunogenic stimulation and/or a defective immunoregulation - they are potentially pathogenic in several ways, e.g. (1) by direct antibody action, (2) by interaction with complement, (3) by antibody-dependent cell-mediated cytotoxicity, and (4) by interaction with phagocytic cells.

Key words
Multiple sclerosis; demyelinating diseases; antibrain antibodies; complement-fixation; IgG subclass; bacterial absorption; myelin protein; galactosylceramide; sulfatide

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STUDIES ON COMPLEMENT-FIXING ANTIBRAIN ANTIBODIES IN MULTIPLE SCLEROSIS

By
BJÖRN RYBERG

Lund 1981

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To Eva,
Per, Anders, Ingrid, and Kristina

This thesis is based on the following publications, which are referred to in the text by their roman numerals:

I Ryberg, B. (1978): Multiple specificities of antibrain antibodies in multiple sclerosis and chronic myelopathy. J. neurol. Sci., 38:357-382.

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III Ryberg, B. (1980): Multiple sclerosis: Search for a serologic inhibitor. Acta neurol. scandinav., 62:193-196.

IV Ryberg, B. (1980): Intrathecal and extrathecal production of antibrain antibodies in multiple sclerosis. J. neurol. Sci., 48:1-8.

V Ryberg, B.: Antibrain antibodies in multiple sclerosis. Relation to clinical variables. Submitted for publication.

VI Ryberg, B.: A longitudinal study of antibrain antibodies in multiple sclerosis. Submitted for publication.

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ABBREVIATIONS

ABA	Antibrain antibody (-ies)
ACTH	Adrenocorticotropic hormone
CM	Chronic myelopathy
CNS	Central nervous system
CSF	Cerebrospinal fluid
EAE	Experimental allergic encephalomyelitis
GMT	Geometric mean titre
Ig	Immunoglobulin
IgG_{SYN}	De novo CNS IgG synthesis (Tourtellotte 1975)
MDH_{50}	50 % haemolysis units
MS	Multiple sclerosis
RA	Rheumatoid arthritis
SD	Standard deviation
SLE	Systemic lupus erythematosus
TLA	"Total lipid antigen"
TLC	Thin layer chromatography

INTRODUCTION

In temperate climates multiple sclerosis (MS) is one of the most important neurological diseases, in some areas reaching a prevalence rate over 200 per 100,000 (Poskanzer et al. 1980). It affects people in their most productive years, leaving a considerable part of them disabled within years or decades (Müller 1949).

This chronic disease involves all parts of the central nervous system (CNS) in an intermittent or steadily progressive way. Its typical lesions, the plaques, have a patchy or confluent distribution along the venules and small veins of the CNS and have a predilection for the white matter (Fog 1965). Histologically, the plaques show a selective loss of myelin and oligodendroglial cells and a proliferation of astroglia (Prineas 1975). In active lesions, the centrally placed venules have collars of lymphocytes, plasma cells, and macrophages (Prineas 1979), and the plaque is surrounded by a zone of numerous cellular elements, the nature of which is debated (Adams 1977). The lesions exhibit firmly bound IgG and complement factors (Lumsden 1971; Woyciechowska and Brzosko 1977) and are often associated with an increased permeability of the blood-brain barrier (Broman 1964; Brown 1978).

The aetiology is unknown, but its uneven geographic distribution, its association with the major histocompatibility complex (Batchelor et al. 1978) and its distribution in twins (Williams et al. 1980) suggest both an environmental and a genetic factor. Attempts to demonstrate a causing agent have failed (Cook and Dowling 1980) and if MS is of infective origin, it has been found likely that it has a long incubation period and that there is a substantial pool of asymptomatic carriers (Acheson 1977).

Evidence of both general and CNS restricted immunological changes in MS has assembled since the demonstration in 1942 by Kabat et al. of increased gamma globulins in the cerebrospinal

fluid (CSF) of MS patients (Aarli and Tönder 1980; Bloom 1980; Lisak 1980). Although antibodies to some common viruses are produced in increased amount (Norrby 1978), the cause of the hyperimmunization in the CNS is not known. The demonstration of a reduced suppressor lymphocyte activity in peripheral blood during certain phases of the disease (Arnason and Antel 1978; Reinherz et al. 1980) and perhaps the presence of lymphocytoxic antibodies (Schochet et al. 1977) provide tentative explanations for some of the immunological changes. Moreover, mechanisms in the brain leading to a local activation or attraction of lymphoid cells and monocytes are proposed to be of importance (Esiri 1977; Vandvik et al. 1979; Cremer et al. 1980). The earliest events in this disease, however, remain completely unknown.

MS is frequently associated with a number of largely uncharacterized humoral factors of possible importance for the manifestations of the disease. Lumsden (1972) and Aarli and Tönder (1980) review the conflicting results of attempts to find autoantibodies to brain constituents in demyelinating diseases. Several immunological methods (complement fixation, indirect haemagglutination, immunofluorenscence, immunoprecipitation, antiglobulin consumption test, radio-immunoassays) have been employed, but the findings are contradictory and difficult to interprete because of an unspecific affinity of normal human IgG to various CNS structures (Aarli et al. 1975; Traugott et al. 1978; Kennedy and Lisak 1981). Gliotoxic and myelinotoxic activities in vitro are abnormalities often found in serum (Berg and Källén 1961, 1962; Bornstein 1963) and CSF (Lamoureux and Borduas 1966) from MS patients. In vivo, MS CSF was myelinotoxic to tadpole optic nerve (Tabira et al. 1977 a), probably by an IgG mediated mechanism (Tabira et al. 1977 b). Further, factors with in vitro neuroelectric blocking properties and of probable IgG nature are frequently found in MS sera (Bornstein and Crain 1965; Schauf and Davis 1978). The importance and interrelation of these factors are still incompletely known.

Working with concentrated CSF, Laurell and Link (1972) found complement fixing antibrain activity in 6 out of 12 patients with MS, but in none of 17 patients without MS. These findings were confirmed and extended in experiments where both CSF and serum were studied (Ryberg 1976). The IgG nature of the CSF reactant was shown and preliminary results on the CNS component (sub-

sequently shown to be just one of several) responsible for the reaction suggested that the phenomenon could be discussed in terms of antibody and antigen (Ryberg 1975).

AIMS OF THE INVESTIGATION

The present study was started after establishing the feasibility of the present experimental approach and the obtaining of results that suggested the involvement of an antigen-antibody reaction (Ryberg 1975, 1976). Its main objectives were:

(1) To characterize the CNS component(s) reacting with serum and CSF from MS patients.

(2) To establish the immunoglobulin nature of the serum and CSF reactant.

(3) To study the relation between antibrain antibodies (ABA) and the oligoclonal immunoglobulins in MS CSF.

(4) To study the distribution of ABA between serum and CSF in order to decide their origin.

(5) To study the occurrence of ABA in MS and other patient groups to enable a discussion on their being a primary or secondary phenomenon.

(6) To study the relation between ABA and clinical variables in order to get a clue to their possible effects in vivo.

SUBJECTS

Patients with MS

The diagnosis of MS was established by the clinical criteria given by Müller (1949) and by Schumacher et al. (1965). In paper V, MS patients were classified as having clinically definitive MS or probable MS according to McDonald and Halliday (1977). The MS patients studied in the various papers belonged to a MS material of about 200 patients treated by the author and are more representative for patients seen at a neurological outpatient department than for the entire MS population. Patients with very mild symptoms and those with severe disability are under-represented. Several patients were included in more than one study. The basis for inclusion in an investigation was non-selective, but the availability of serum and CSF in the individual patients might have introduced an unintentional bias.

In paper V MS patients were classified according to degree of disability employing the criteria used by Olsson et al.(1976). Patients with mild symptoms that to a slight extent or not at all influenced their professional and social life were classified as not disabled. Those with symptoms that affected professional or social life were classified as disabled. The course of the disease was categorized as described by Källén et al. (1975): (1) repeated relapses, but complete or nearly complete remissions and thus no persisting deficit; (2) repeated relapses with neurological sequelae; (3) steadily progressive course after initial relapses. Patients with a remittent course were subdivided according to clinical activity: (A) no clinical activity of the disease from four weeks before to two weeks after sampling; (B) exacerbation during this time.

Patients with chronic myelopathy

Serum and CSF from fifteen patients with chronic myelopathy of undetermined cause, a heterogenous condition often identified as MS (Marshall 1955, Poser et al. 1978), were studied in paper I.

Patients with other diseases

In papers I and V, control materials consisting of samples from patients with a variety of neurological diseases mostly involving the CNS were included. None of them were considered to have MS. In paper III, sera from 12 patients with various neoplasms and 11 patients with rheumatoid arthritis were studied.

"Healthy" controls

Serum and CSF samples from patients investigated for minor complaints and found not likely to have organic disease were studied in papers I and V.

MATERIALS

Cholesterol, lecithin (synthetic and from soy bean), sphingomyelin from sheep erytrocytes, and glucosylceramide from Gaucher's spleen were from commercial sources. Lecithin from egg yolk was kindly supplied by Dr Björn Åkesson. Purity of these substances was assessed by thin layer chromatography (TLC). Galactosylceramide and sulfatide with normal and hydroxy fatty acid residue were prepared as described below.

Rabbit antisera to human albumin, IgG (specific for γ-chains), and IgM (specific for μ-chains) were purchased from Dako-immunoglobulins a/s (Copenhagen, Denmark).

METHODS

As a consequence of the experimental feed-back situation, some of the methods were subjected to minor adjustments between the separate sections of this investigation. However, the procedures described below should be representative.

Serum and CSF

Serum and CSF were as a rule obtained at the same time. Fifteen to 25 ml CSF were drawn by lumbar puncture. After cell counting by phase contrast microscopy (Sörnäs 1967) and centrifugation, the CSF specimens were heated to 56°C for 30 min together with the serum samples. CSF was concentrated about 100 times by ultrafiltration in a collodion bag (Sartorius, Membranfilter GmbH, Göttingen, FRG) at +4°C as described by Mies (1953).

Tissue samples

Human tissue samples without macroscopic disease involvement were obtained at autopsy 12-36 hours after death from patients without disseminated neoplasm, infection, or systemic disease. Brain tissue was obtained from one patient with a clinical and post-mortem diagnosis of MS and gastric carcinoma. Bovine and rat brains were obtained shortly after the killing of the animals. The tissues were stored at -20°, -70°, or -100°C until further prepared.

Quantitation of proteins

Albumin, IgG, and IgM were quantitated by single radial immunodiffusion (Mancini et al. 1965) using pooled serum from healthy blood donors as standard.

Evaluation of the blood-brain barrier and intrathecal IgG production

The condition of the blood-brain barrier was evaluated by the CSF/serum albumin ratio (Link and Tibbling 1977). Intrathecal IgG synthesis was studied by means of the IgG-index (Delpech and Lichtblau 1972; Tibbling et al. 1977) and by the formula of Tourtellotte (1975) (IgG_{SYN}).

Agar gel electrophoresis

Electrophoresis in agar gel was performed as described by Wieme (1959). Serum and concentrated CSF were applied in an amount corresponding to 5 µg IgG/1 mm of the application through (Link 1973).

Gel filtration

Gel filtration on Sephadex G-200 Superfine and Sepharose 4B was performed by standard procedures.

Isoelectric focusing

Isoelectric focusing was performed on PAGplates ^{R} (LKB, Stockholm, Sweden), pH range 3.5-9.5, as recommended by the manufacturer. After focusing for 90 min, the plates were stained with Coomassie Brilliant Blue, following the manufacturer's instructions.

Preparation of myelin

Myelin was prepared from fresh human white matter as described by Hallpike (1972); its purity was assessed by electron microscopy.

Lipid extraction

The method of Folch-Pi (1972) was used to obtain lipid extracts.

Removal of glycerol ester lipids

A batch of brain lipids free from glycerol ester lipids was prepared by mild alkaline hydrolysis as described by Karlsson et al. (1973).

Thin layer chromatography (TLC)

Lipid samples were dissolved in chloroform-methanol (2:1, v/v) and applied to 20 x 20 cm glass plates coated with a 0.5 mm layer of Silica gel H, type 60 (Merck, Darmstadt, FRG) that had been heat activated. The plates were usually developed in chloroform-methanol-water (65:25:4, v/v/v) (Kates 1972). Analytical plates were charred at 180°C after spraying with a sulphuric acid dichromate spray. Lipid fractions of interest were recovered as described by Åkesson et al. (1970) after preparatory TLC.

Preparation of cerebroside and sulfatide

Highly purified fractions of cerebroside and sulfatide were obtained by column chromatography on silicic acid of a glycolipid extract from human brain (Hakomori and Siddiqui 1974) followed by repeated runs of TLC at different pH. Subfractions of these glycolipids representing subtypes with normal and hydroxy fatty acid residue were isolated.

Storage of lipids

Lipids were kept dissolved in chloroform-methanol (2:1, v/v) at - 20°C.

Antigen preparations

Initial studies (paper I) were performed using a crude extract of human brain obtained by homogenization of grey and white matter in saline, followed by low-speed centrifugation and recovery of the supernatant. Subsequently (papers I to VI) whole homogenates of tissue from brain and other organs were employed in the complement fixation test in order to get more comparable antigen preparations. Usually tissues were homogenized in cold saline in a ratio of 1:50 or 1:100 (w/v).

The techniques of Rapport and Graf (1967) for the preparation of lipids for immunological studies were followed. A cholesterol-enriched total lipid antigen (TLA) from different organs was obtained by evaporation under nitrogen of an amount of lipid extract with an additive of cholesterol. The dried lipid mixture was dissolved in 0.1 ml ethanol and 0.9 ml of 0.9 % NaCl was added. Before use as an antigen, this preparation was further diluted in saline. The optimal proportions of the lipids were empirically found.

Fractions of brain lipids were prepared for the complement fixation test in a similar way by the addition of lecithin and cholesterol. These auxiliary lipids were generally employed in the proportions 1:1 or 1:3,w/w). Because of anticomplementary activity, some of the lipid antigen preparations had to be diluted.

Absorptions with brain constituents

Absorption of serum and CSF with tissue homogenates or myelin suspensions were usually performed for 1 hour at 37°C and overnight at +4°C. The supernatants were collected after centrifugation.

Bacterial absorptions

Absorptions of ABA containing samples were performed with the following heat- and formaldehyde stabilized bacteria with well characterized immunoglobulin-binding properties: Staphylococcus aureus strainCowan I (NCTC No 8530), group A streptococcus strains AR 1, and AW 43, group G streptococcus strain G 148, and Staphylococcus epidermidis strain L 603 (Kronvall et al. 1979). The immunoglobulin binding characteristics of these strains are shown in Table 1.

Table 1. Immunoglobulin binding characteristics of the bacterial species employed in this study

	IgG_1	IgG_2	IgG_3	IgG_4	IgA	IgM
S. aureus Cowan I	+	+	-	+	(±)	(±)
Streptococcus AR 1	+	+	+	+	-	-
Streptococcus AW 43	(+)	(+)	(+)	(+)	+	-
Streptococcus G 148	+	+	+	+	-	-
S. epidermidis L 603	-	-	-	-	-	-

Complement fixation test

The complement fixation test was performed on plexiglass plates with U-shaped wells according to the micromethod of Fulton and Dumbell (1949). Five 50 % haemolysis units (MDH_{50}) of guineapig complement were employed and a photometrically standardized 0.2 % suspension of sheep erythrocytes sensitized with 6 MHD_{50} of amboceptor served as haemolytic system. The reaction was carried out at +4°C overnight, and after the addition of sensitized sheep erythrocytes, the plates were incubated at 37°C for two hours before reading. Fifty per cent or less haemolysis, visually estimated, was recorded as a positive reaction. Controls for anticomplementary activity in antigen preparation, serum, and CSF were included. Veronal buffer (pH 7.2) containing 0.15 mmol/l Ca^{2+} and 0.5 mmol/l Mg^{2+} was used throughout the complement fixation experiments.

Intra- and inter-experimental variation of results obtained with this test were determined in papers V and VI. Neither exceeded two dilution steps. However, a greater variation when different batches of reagents are used over a longer time, cannot be ruled out.

Two definitions of 'titre' are prevalent in the immunological literature. In papers II, III, and IV 'titre' was used in the sense of the end point dilution or end point concentration of serum and CSF giving a positive reaction. In subsequent work (papers V and VI) it was found preferable to use the other definition of 'titre', i.e. the reciprocal of the end point dilution or concentration.

Statistical methods.

The significance of differences in routine laboratory variables was checked by Student's t-test. Differences in titres were assessed using the Mann-Whitney U-test. Proportions were compared by Fisher's exact test or the chi-square test. Correlations were calculated using Pearson's and Spearman's coefficients. Where multiple comparisons are performed within the same groups, the risk of finding significant differences or correlations by chance should be observed.

Samples in which titres could not be determined because of anticomplementary activity were excluded from most statistical calculations.

RESULTS AND DISCUSSION

A. Characterization of CNS components reacting with MS samples

(Paper I)

Although antibodies to gangliosides (Yokoyama et al. 1962; Hirsch and Parks 1976) galactocylceramide (Dupouey 1972; Hirsch and Parks 1976), and myelin basic protein (Sheremata et al. 1978; Panitsch et al. 1980) have been reported in sera from MS patients and other subjects, the specificity of complement-fixing ABA in MS has not been studied before.

Paper I presents the results from experiments performed to characterize the CNS components (here discussed as antigens) involved in the complement fixation reaction with serum and CSF from MS patients. Samples for this work were mainly obtained by screening serum and CSF from 60 MS patients, employing a crude saline extract of human brain and an cholesterol-enriched lipid extract of human brain (TLA) as antigen preparations.

The tissue distribution of antigens responsible for the positive reactions between MS samples and the saline brain extract was studied by testing homogenates of a large number of human tissues. In particular, several regions of the nervous system were assayed for antigenic activity, as was also myelin from human white matter. Bovine brain and, in some instances, rat brain served as control for species specificity.

The size of the antigen carrying structures was estimated by aid of gel filtration on Sepharose 4 B, which has an exclusion limit at a molecular (particle) weight of several million daltons. As all antigenic activities were eluted in the void volume, it was concluded that they were not in true solution in the aqueous media studied.

All nonlipid antigens were inactivated by heating to 80°C for 10 min.

Serum and CSF samples reacting with TLA were further

studied by testing against successively purer lipid fractions obtained by mild alkaline hydrolysis and preparatory TLC. The individual chromatographic fractions usually did not react with any of the samples that reacted with TLA, but by the systematic addition of varying amounts of auxiliary lipids (usually lecithin-cholesterol 1:1 or 1:3, w/w), reactivity with a few MS samples could be restored and traced to two defined lipid fractions. They were identified as galactosylceramide and sulfatide (galactosylceramide-3-sulfate). Despite their structural similarity, no cross-reactivity was found between these antigens (haptens), nor with glucosylceramide (Fig. 1).

Antibody to galactosylceramide has not earlier been reported in human CSF. Antibody to sulfatide has not previously been documented in man, but can be induced in experimental animals (Hakomori 1974).

Reactivity against the lecithin-cholesterol mixture without added hapten was found in some MS and non-MS samples. Similar reactivity was found in several other lipid species in combination with cholesterol. The basis for this reactivity is unknown.

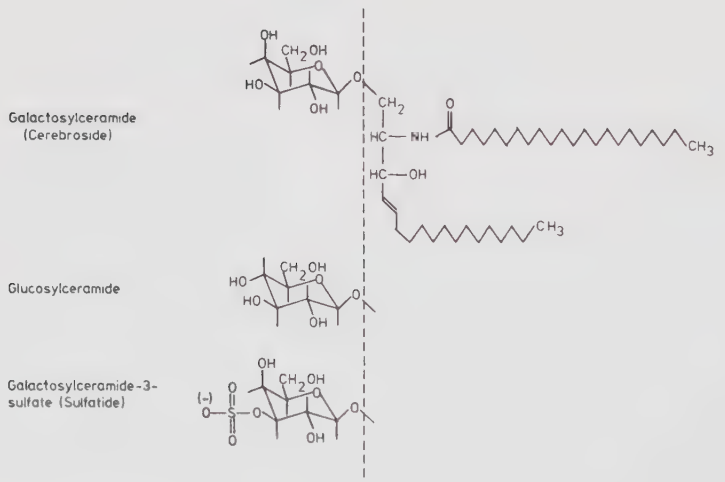

Figure 1. Structure of the three glycosphingolipid haptens studied. Polar head groups are to the left. To the right a common ceramide moiety is represented.

From the findings with individual MS samples, at least seven more or less organ-specific antigens or antigen-like activities in human CNS were inferred:

I. Antigen associated with CNS myelin. Organ specific. Labile to heat. Not solubilized by saline or distilled water. Nonlipid. Not species specific.

II. Antigen with the properties of antigen I except for species restriction (found in human and rat brain, but not in bovine brain).

III. Equally distributed between white and grey matter. Organ specific. Labile to heat. Not solubilized by saline. Nonlipid. Not present in myelin.

IV. Antigen present in white and grey matter, but predominantly in grey. Probably also present in neurohypophysis and epiphysis and possibly in some nonneural tissues. Labile to heat. Not solubilized by saline. Nonlipid.

V. Galactosylceramide. Relatively specific for myelin. Heat resistant. Varying auxiliary lipid requirements.

VI. Sulfatide (galactosylceramide-3-sulfate). Relatively myelin specific. Stabile to heat.

VII. Cholesterol in combination with a variety of lipids.

Several of these antigens might prove to be heterogeneous and further specificities will probably be delineated by future research. Antibody to antigen I was found in nearly half of the MS patients, whereas antibodies to each of the other six antigens were demonstrable only in a few patients. No general tendency for the simultaneous presence of ABA of more than one specificity in the same patient was apparent in the studied material.

All the antigens reacting in saline extract of human brain (antigens I to VI) have been demonstrated in a large number of human brains, including one MS brain. In fact, no adequately stored human brain sample has given negative reactions with MS samples with known ABA content. Thus there is no reason to believe that the reactions depend on alloantigens. MS brain did not yield additional positive reactions when eight serum and CSF samples non-reactive with normal human brain were tested. The present findings therefore do not completely support the results of Sachs and Steiner (1934). Employing a complement fixation technique and alcoholic extracts of formalinized human CNS, these authors found MS brain to be superior to non-MS brain as

antigen in the reaction with MS samples. The difference between MS brain and non-MS brain in the study of Laurell and Link (1972) was marginal. Although antigenic differences between MS brain and normal brain seems to exist (Rastogi et al. 1978), these antigens are evidently not involved in the reactions studied here.

B. Antigen studies in developing mouse brain (Addendum)

Developing mouse brain is a useful model for the study of CNS specific antigens, as myelination takes place in the postnatal period (Jacque et al. 1976). This fact was employed in an attempt to get further information on the specificities of complement-fixing ABA (Ryberg 1977, unpublished). No or very low antigenic activity was found in homogenates of brain from newborn mice of an inbred laboratory strain, when tested against MS sera representing ABA specificities I, II, IV, and VI. All the corresponding antigens exhibited a steep increase in activity (titre/g of wet brain tissue) from day 5 to day 25 of the postnatal period. The increase in activity for antigens I and II was during this time at least four-fold and for antigens IV, and VI about 16-fold. The concentration (w/w) of protein (soluble and non-soluble in saline) only doubled in the same period. The ontogenetic pattern in mouse for these antigens is shared by several known brain constituents (Jacque et al. 1976) and the lacking discriminating value of these experiments did not encourage further work in this field.

C. Antigen studies in mutant mice (Addendum)

A number of strains of mutant mice with defects in the formation and maintenance of myelin have been intensely studied during recent years (Bunge 1980). Among the best characterized are the jimpy mice with an X-linked recessive defect and quaking mice with an autosomal recessive defect. The existence of a number of quantitative biochemical abnormalities in these mutants (Hogan 1977) was utilized to gather information on the antigens reacting with ABA in MS samples (Ryberg and N. Baumann, unpublished). Brains of 28 $\pm$ 1 days old jimpy and quaking mice and their littermate controls were homogenized in cold veronal buffer in the proportions 1:19 (w/v). For comparison, homogenates of human white and grey matter were similarly prepared. The

Table 2. Antigenic activities in brains of jimpy and quaking mice, their littermate controls, and in human white and grey matter. End point dilutions of brain tissue in veronal buffer (w/v) are given.

	Antibody Specificity	Jimpy		Quaking		Human	
		Mutant	Control	Mutant	Control	White matter	Grey matter
Serum (1:4)							
1	I	1:20	1:40	1:20-40	1:40	1:640-1260	1:320-640
2	I	1:20	1:80	1:40-80	1:80	1:640	1:320
3	II	1:20	1:80	1:40	1:80	1:320	1:80
4	IV	1:320	1:80-160	1:160	1:160	1:20	1:160
5	VI	1:<20	1:40	1:<20	1:40	1:5120	1:320
CSF (10:1)							
6	I	1:<20	1:80	1:<20	1:40	1:640	1:320
7	I	1:<20	1:160	1:20	1:160	1:5120	1:2560
8	I	1:<20	1:160	1:<20	1:80	1:2560	1:1280
9	II	1:20	1:40	1:20	1:40	1:320	1:160

homogenates were titrated against a number of MS samples with partly characterized ABA. Preliminary results are presented in Table 2.

The findings confirm the heterogeneity of ABA. Also within the earlier delineated specificity I, some heterogeneity is seen. Whether or not this depends on multiple antibody specificities within the individual patients remains to be decided. In agreement with observations in paper I, the findings suggest an association of antigens I, II, and VI to the myelin-oligodendroglia unit.The higher activity of antigen IV in jimpy brain than in control brain is perhaps an effect of gliosis. The two antigens, glia fibrillary protein and D5 protein, which are increased in jimpy brain (Jacque et al. 1976), should be interesting candidates in the identification of antigen IV.

D. Partial characterization of antigen I (Addendum)

Antigen activity of type I in human brain has been extensively studied by aid of MS sera with the corresponding reaction characteristics (Ryberg, unpublished). The antigen has the properties of an intrinsic membrane protein which is partly exposed on the surface to allow the reaction with antibody (ABA). Briefly, this is seen from its firm association with the particulate fraction of white matter homogenates even under the influence of low and high (3 M NaCl, 3 M KCl) ionic strength, low and high pH (4.0-11.0), chaotropic ions (3.5 M KSCN), reducing agent (3 M 2-meracptoethanol), and chelating agent (0.1 M Na_2 EDTA). The protein nature of the antigen, suspected from its temperature lability (Paper I), was established by inactivation by proteolytic enzymes (trypsin, chymotrypsin, papain, and pronase). This inactivation was a slow process at room temperature, but could be considerably speeded up by the addition of a small amount of a non-ionic detergent, e.g. Triton X-100 to the white matter suspension, or by heating the suspension to 60°C for 10 min before enzymatic treatment. The antigenic activity does not seem to depend on a carbohydrate epitope, as it resisted extensive treatment by neuraminidase, ß-galactosidase, and α-mannosidase and was not inactivated by 1 mM potassium periodate (Parish et al. 1976).

E. Immunoglobulin characteristics of ABA (Papers I and II)

IgG and IgM are the only immunoglobulin classes fixing complement upon antigen binding. This was utilized to characterize ABA in paper I. Five MS sera reacting with the saline brain extract and three MS sera reacting with TLA were separated by gel filtration on Sephadex G-200. The fractions obtained were assayed for IgG, IgM, and ABA (cf. Fig. 1, paper I). In all sera, ABA activity coeluted with IgG. However, one sequentically studied patient had a transient occurence in serum of ABA of the IgM class, in addition to a slowly increasing titre of IgG antibodies. This finding suggests the effect of a fresh antigenic stimulation.

A different approach was tried in paper II. Five serum and six CSF samples from 11 MS patients were absorbed with bacteria with specific surface receptors for various immunoglobulins. As none of the presently available bacterial strains has an exclusive affinity for immunoglobulins (Kronvall, personal communication), it was imperative in this study to use a number of strains with different absorption characteristics (Table 1).

The MS samples were known to contain ABA directed against CNS antigens I to IV as defined in paper I. Reactivity with brain homogenates was eliminated in all 11 samples by absorption with one or more of the following IgG absorbents: S. aureus Cowan I and streptococcus strains AR 1 and G.148. Compared with aliquots absorbed with S. epidermidis L 603 with no affinity for IgG, the reduction in ABA titres was significant ($\geq$ 2 titre steps), and the decrease in IgG concentration was >90 %. Absorption with streptococcus strain AW 43, with low affinity for IgG, was not effective in eliminating antibody.

The results indicate that ABA were of IgG class in all the tested samples. Of the IgG subclasses, only 1, 2, and 3 are complement-fixing (Spiegelberg 1974). The efficient absorption of ABA by S. aureus Cowan I carrying protein A, therefore indicates that they are usually of IgG_1 and/or IgG_2 subclass. IgG_1 is of particular interest in this context as this subclass usually is most elevated in MS CSF (Palmer et al. 1976, Vandvik et al. 1976, Eikhoff et al. 1979).

The complement fixing ABA are not identical with the principal in vitro demyelinating factor in MS serum. Grundke-Iqbal and Bornstein (1979) were unable to eliminate this factor

by protein A absorption and have recently demonstrated its non-immunoglobulin nature (Grundke-Iqbal and Bornstein 1980).

F. Relation of ABA to oligoclonal IgG in CSF (Addendum. Paper V)

Although a minor part of the oligoclonal IgG in MS CSF is reported to be absorbable by measles antigens (Norrby and Vandvik 1975) and staphylococcal lipoteichoic acid (Aasjord et al. 1981), the specificity of its greater part remains unidentified.

The ability of human brain constituents to absorb out ABA and oligoclonal bands in MS samples has been studied (Ryberg and K.Nilsson 1978, unpublished). Aliquots of eight MS sera and 12 concentrated MS CSF samples containing ABA of specificities I to VI (paper I) were subjected to absorption with myelin or other particulate fractions of human brain under conditions known to remove ABA. Absorbed and non-absorbed aliquots were separated side by side by isoelectric focusing on PAGplates ^{R}, pH range 3.5-9.5, and stained with Comassie Brilliant Blue.

In no sample did the absorption cause a reproducible loss or reduction of any of the numerous bands in the IgG containing alkaline region or elsewhere. The results were interpreted as evidence that no individual oligoclonal band, separable by this high-resolving technique, consisted of only ABA. The clonal distribution of ABA must evidently be studied by more sensitive methods. One such attempt (Shorr et al. 1981) employed imprint electroimmunofixation (Nordal et al. 1978). The negative result attests to the quantitatively unimportant nature of ABA in MS, but does not provide any qualitative information.

No correlation between ABA in serum and CSF, on one hand, and the presence of oligoclonal bands in CSF after separation on gel eletrophoresis, on the other, was in paper V found in patients with clinically definitive MS. These variables therefore do not seem to provide synonymous information.

G. Experiments with samples lacking demonstrable ABA (Paper III)

A factor able to interfere with the antigen-antibody reaction or complement fixation would complicate the evaluation of results obtained with the complement fixation technique. Factors of this kind are known in the immunological literature

and, notably, rheumatoid factors are potential inhibitors of the complement fixation reaction (Heimer and Levin 1965; Riski et al. 1977).

Using a modification of the method for the demonstration of ABA, experiments were performed to reveal unspecific serologic inhibiting activity in serum and CSF samples without demonstrable ABA. None of 15 serum samples and 11 CSF samples from 20 MS patients showed any inhibiting activity. This, however, was found in a number of sera from patients with rheumatoid arthritis and malignant neoplasms. Failure to detect complement-fixing ABA in MS therefore is not likely to depend on in vitro inhibiting factors.

H. Distribution of ABA between serum and CSF (Paper IV)

Numerous findings suggest in MS a local hyperimmunization in the CNS. Perivascular cuffs of lymphocytes, plasma cells, and macrophages are seen in brain tissue (Prineas 1979). IgG in CSF Lowenthal 1964; Link 1967) and CNS (Link 1972; Mattson et al. 1980)is of an oligoclonal nature, and there are indicators of an intrathecal IgG production (Frick and Scheid-Seydel 1958; Delpech and Lichtblau 1972; Sandberg-Wollheim 1974). Moreover, in the absence of signs of an active infection, antibodies to several common viruses are often found to be synthesized within the CNS (Norrby et al. 1974).

A wide range of CSF/serum ratios was found when complement-fixing ABA of several specificities were titrated in paired serum and CSF samples from 27 MS patients. Most ratios were higher than known ratios for a number of reference antibodies (Norrby et al. 1974; Fossan 1977), the normal CSF/serum IgG ratio (0.002 to 0.003), and the CSF/serum IgG ratios in the individual patients. When the ratio (CSF/serum ABA) ÷ (CSF/serum albumin) was formed by analogy with the (CSF/serum IgG) ÷ (CSF/serum albumin) ratio (Delpech and Lichtblau 1972) to compensate for a possible impairment of the blood-brain barrier, a wide range of values was found. From these and further considerations it was found likely that ABA are produced on both sides of the blood-brain barrier in proportions that vary greatly between the individual patients. Therefore, the responsible MS-related immunological aberration may not be limited to the CNS, but

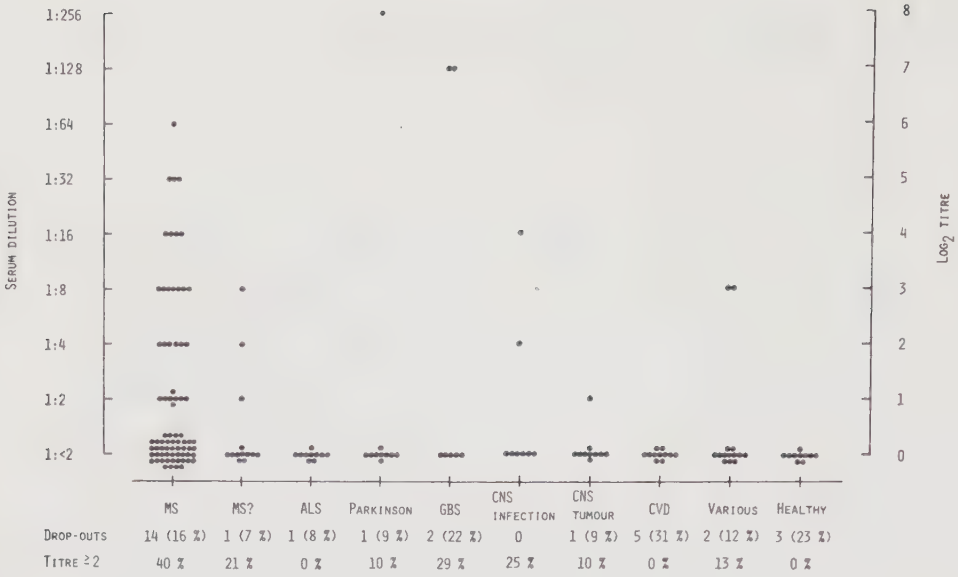

Figure 2. Results of ABA titrations against brain homogenate of serum from various patient groups and healthy controls. MS = clinically definitive MS, MS? = probable MS, ALS = amyotrophic lateral sclerosis, GBS = Guillain-Barré syndrome, CVD = cerebrovascular disease.

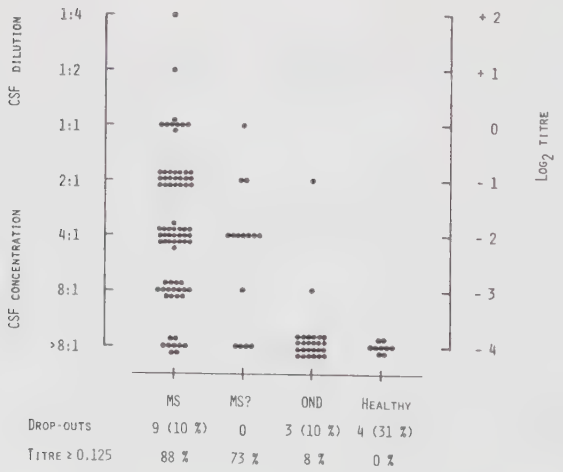

Figure 3. Results of ABA titrations against brain homogenate of CSF from various patient groups. Abbreviations as in Fig. 2. OND = other neurological disease.

often seems to include the extrathecal compartment. In papers V and VI a slightly rising trend for ABA titres during the long term course of the disease was found in serum but not in CSF. This might reflect a progressive dissemination of the autoimmune process beyond the CNS.

ABA are unique in being principally directed against and probably being induced by CNS specific antigens within a relatively secluded compartment. The probable synthesis of ABA also outside the CNS-CSF compartment may be explained by the release of immunogenic CNS material to the general circulation through the arachoidal villi or to the cervical lymph nodes by the as yet little known routes studied by Bradbury and Cole (1980). Another possibility would be the egress of lymphocytes sensitized within the CNS.

The metabolism of ABA is not known; if it differs from that of IgG in general, the above conclusions may have to be revised. It should be mentioned here that autoantibodies in some diseases have a tendency to be absorbed in the target organ.

I. ABA in MS and other diseases (Papers I and V)

Despite some claims of the contrary, no single clinical or laboratory test specific for MS seems to exist. Oligoclonal IgG and other evidence of intrathecal IgG synthesis is for example found in CNS infections (Link and Müller 1971), and the largely uncharacterized in vitro gliotoxic and demyelinating factors described in MS by Berg and Källén (1961), by Bornstein (1963), and by others are found in conditions not related to MS. In particular, the frequent finding of such factors and of immunoglobulins binding to myelin (Lisak et al. 1975) in amyotrophic lateral sclerosis is remarkable, as this disease is not characterized by a primary demyelination.

It is therefore of considerable interest that complement-fixing ABA assayed against brain homogenate, were relatively specific for MS (Figures 2 and 3). The absence of ABA in sera from patients with amyotrophic lateral sclerosis is notable.

The finding of ABA reacting with CNS myelin in two of nine sera from patients with the Guillain-Barré syndrome confirms reports of Melnick (1968) and Nyland and Aarli (1978). The discordant reaction with brain lipids in the two patients

probably implicates a heterogeneous autoimmune response in this disease, similar to the situation in MS (paper I).

In paper I a high prevalence of ABA was found in chronic myelopathy, a heterogeneous condition often identified as MS (Marshall 1955; Poser et al. 1978).

Except in MS, chronic myelopathy and the Guillain-Barré syndrome, ABA against brain homogenates were found in single cases of SLE, spinal meningioma, Parkinson's disease, neurosyphilis, malignant glioma, epilepsy, and subacute combined degeneration of the spinal cord. These disorders have not been studied in a number sufficient to estimate the prevalence of ABA, and the specificities of the found antibodies are largely unknown. They might well be different from those found in MS, as seen from the cases not reacting with CNS myelin. Antibodies against brain lipids appear less specific for MS than those against nonlipid brain antigens. From the great number of negative findings in control patients with definitive CNS pathology, it is clear that the mere destruction of CNS tissue is not a sufficient prerequisite for the induction of ABA in detectable amount.

The relative specificity of ABA for demyelinating diseases might have a diagnostic value that will probably be enhanced by work with selected CNS antigens.

J. Relation of ABA to clinical variables in MS (Paper V)

In spite of a clear tendency in the 87 patients with clinically definitive MS studied in paper V, for male patients to have more pronounced abnormalities in the routine laboratory variables (CSF mononuclear cell count, CSF-IgG, serum-IgG, CSF/serum albumin, IgG-index, and IgG_{SYN}), there was no significant difference in ABA titres between the two sexes.

There was a tendency towards higher CSF ABA titres in younger patients and in those with an earlier onset of disease. ABA were rarely found in serum in patients with a very short duration of MS (classified as early probable MS).

A comparison of patients matched for the time variables disclosed that those with a chronically progressive course had clearly higher ABA titres (serum GMT 150 % higher, $P < 0.05$; CSF GMT 97 % higher, $P > 0.05$) than those with a remittent course. The differences in ABA titres were greater than for any of the studied routine laboratory variables.

No significant difference was found in ABA titres or routine laboratory variables between patients in exacerbation and those in remission.

Disabled patients tended to have higher ABA titres than matched (and nonmatched) non-disabled MS patients. GMT of CSF ABA in patients reaching disability during the period 15-20 years after the onset of MS was 180 % higher than in matched patients still not disabled after 20 years ($P < 0.05$, Mann-Whitney U-test). This difference is much greater than for any other variable studied.

All MS patients in whom both serum and CSF titres against brain homogenate were known are displayed in Fig. 4. The CSF/serum ABA ratio can be read by aid of the diagonal lines in all patients where both titres were above baseline. There was a weak

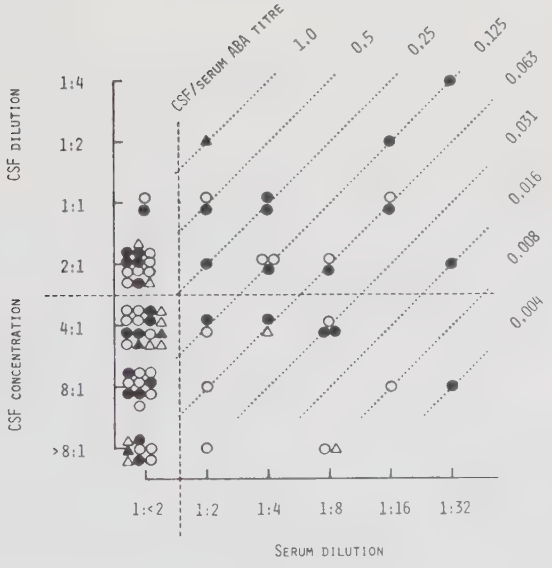

Figure 4. The relation between ABA titres in serum and CSF in MS patients. The dotted diagonal lines give the CSF/serum ABA ratios for paired serum and CSF titres. ○ = clinically definitive MS, not disabled. ● = clinically definitive MS, disabled. △ = probable MS, not disabled. ▲ =probable MS, disabled. The interrupted lines subdivide the patients into four groups: (a) those with a low serum titre and a "high" CSF titre, (b) those with "high" serum and CSF titres, (c) those with low serum and CSF titres, and (d) those with a "high" serum titre and a low CSF titre.

Table 3. Characterization of patients with clinically definitive MS subgrouped according to ABA titres in serum and CSF (Fig. 4)

Results are given as number and percentage or as means ± SD

	a n = 13	b n = 15	c n = 25	d n = 11
Male	8[a] (62 %)	5 (33 %)	7 (28 %)	3 (27 %)
Female	5 (38 %)	10 (67 %)	18 (72 %)	8 (73 %)
Clinical course 1	5 (38 %)	1[b] (7 %)	10 (40 %)	3 (27 %)
Clinical course 2	5 (38 %)	6 (40 %)	7 (28 %)	5 (45 %)
Clinical course 3	3 (23 %)	8 (53 %)	8 (32 %)	3 (27 %)
Disability +	6 (46 %)	10 (67 %)	10 (40 %)	5 (45 %)
Disability -	7 (54 %)	5 (33 %)	15 (60 %)	6 (55 %)
Clinical activity A	7 (54 %)	5 (33 %)	12 (48 %)	6 (55 %)
Clinical activity B	3 (23 %)	2 (13 %)	5 (20 %)	2 (18 %)
Age (yr)	39.5 ± 9.3	39.7 ± 7.5	42.6 ± 9.1	44.7 ± 14.3
Onset (yr)	26.1 ± 7.8	27.7 ± 7.8	30.7 ± 9.1	27.8 ± 8.8
Duration (yr)	13.4 ± 9.3	12.3 ± 7.2	11.8 ± 6.8	16.7 ± 8.4
CSF mononuclears[e]	13.0 ± 22.1	9.2 ± 15.1	6.2 ± 5.1[c]	2.7 ± 1.5
CSF-IgG (g/l)	0.094±0.064	0.088±0.039[c]	0.061±0.030	0.052±0.027
Serum-IgG (g/l)	11.0 ± 1.9	10.1 ± 2.0	9.7 ± 2.0	9.4 ± 3.9
CSF/serum albumin	5.2 ± 2.2	5.5 ± 1.8[d]	4.3 ± 1.6	4.5 ± 1.8
IgG-index	1.8 ± 1.2	1.6 ± 0.6	1.6 ± 0.7	1.2 ± 0.7
IgG_{SYN} (mg/d)	30 ± 30	28 ± 18	18 ± 13	12 ± 11

a Proportion of males higher than in c and in b + c + d, $P < 0.05$ (Fisher's exact test)

b Proportion of clinical course 1 lower than in a + c + d, $P < 0.05$ (Fisher's exact test)

c Higher than in d, $P < 0.05$ (Student's t test)

d Higher than in c, $P < 0.05$ (Student's t test)

e Cells x 10^6/l

positive correlation between serum ABA and CSF ABA. None of the CSF/serum albumin ratios were much elevated (highest value 0.0113), indicating a very limited access of serum antibody to CSF. The wide range of CSF/serum ABA ratios therefore probably reflects varying proportions of intrathecal and extrathecal antibody synthesis (paper IV).

Subdivision of the clinically definitive MS patients according to Fig. 4 into four groups revealed an interesting pattern (Table 3). Patients in group a were predominantly male and some had a marked mononuclear pleocytosis in the CSF, high CSF-IgG, high IgG-index, and high IgG_{SYN}, giving elevated mean values of these variables. Also in group b many patients had high CSF mononuclear cell counts and high CSF-IgG levels. Moreover, relatively high CSF/serum albumin ratios were found in this group indicating some impairment of the blood-brain barrier. This agrees with the high proportion of disabled patients (67 %) and malignant courses ("2" and "3" comprising 93 %) in this group with high serum and CSF ABA titres. Patients in group c, which had the lowest proportion of disabled (40 %), had somewhat lower CSF mononuclear cell counts and IgG parameters in CSF than the whole MS group. This tendency was much more marked in group d where patients were generally somewhat older and had a longer mean duration of disease than in groups a, b, and c.

The interesting association between ABA, on one hand, and a more malignant course and disability, on the other, raises the important question whether they are pathogenic or merely an epiphenomenon of the disease.

K. A longitudinal study (Paper VI)

In a longitudinal study of 35 patients followed for up to five years, ABA in serum was assayed; in 18 of them also CSF titres were determined. Altogether, 281 sera and 144 CSF samples were tested. Low titres or anticomplementary activity invalidated some observations and many titres were obtained at times when they could not be related to a single, well-defined clinical event. However, enough observations were at hand to make clear that ABA titres in serum and CSF are essentially stable during the course of MS. No significant change in ABA titres occurred in close relation to several clinical exacerbations (Figures 5 and 6). This finding does not favour the idea of an active regulatory role for ABA in these clinical events. However, the MS

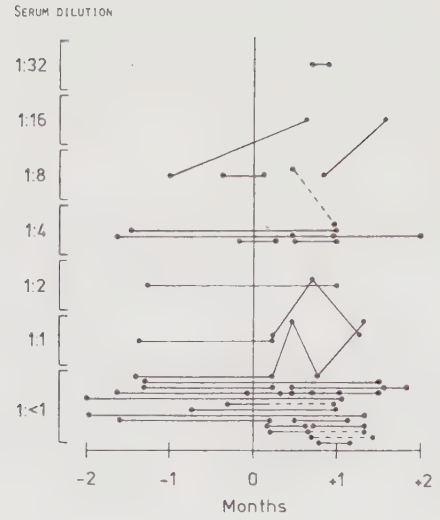

Fig. 5: Serum titre of ABA in exacerbating MS. All observations obtained in two or more samples within the indicated period before and/or after an attack are included. Excluded are single observations and titres obtained during a previous bout or within four weeks after its subsidence or within six weeks after the termination of an ACTH course. An interrupted line indicates that an ACTH course has been instituted.

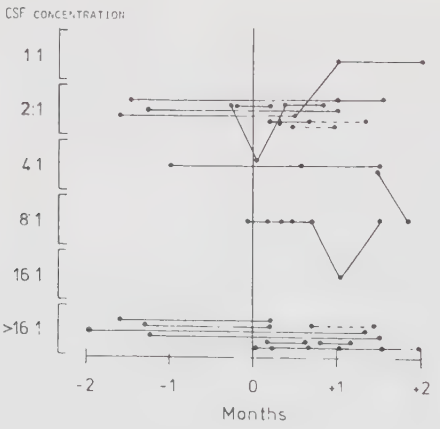

Fig. 6: CSF titre of ABA in exacerbating MS. For explanations, see Fig. 5.

lesions are anatomically limited, each plaque appearing to have its individual IgG composition (Esiri 1980; Mattson 1980) and a change in the concentration or properties of ABA in the local environment of an active plaque is perhaps not reflected in serum or lumbar CSF.

The absence of fluctuations correlated to the clinical events do not rule out a pathogenic role for ABA. Harmful effects of such antibodies could be temporally and anatomically restricted by a number of factors, e.g., the condition of the blood-brain barrier and the availability of humoral and cellular elements able to co-operate with the antibodies.

The relative stability during and after relapses, which ABA share with most other antibodies studied, suggests that the drop in suppressor cells in blood during MS attacks (Reinherz et al. 1980) is not sufficient to cause a general effect on humoral immunity. Because of the possible role of tissue destruction for the induction of ABA, it is of interest that no separate bout gave a significant boosting of ABA.

GENERAL DISCUSSION

The concept of an autoimmune pathogenesis in MS has received much inspiration from the successful work with experimental allergic encephalomyelitis (EAE) (Paterson et al. 1981). A satisfactory pathogenetic model, however, has yet to be found.

The conflicting nature of early attempts to demonstrate circulating antibrain antibodies in MS is evident from the review by Lumsden (1972), although this author had the impression "that such work was on the fringe of an authentic phenomenon". Also more recent claims of autoantibodies in MS to CNS constituents (Abramsky et al. 1977) have been challenged (Traugott et al. 1979; Kennedy and Lisak 1981). One explanation for these inconsistencies is the unspecific affinity of normal human IgG to various CNS structures (Allerand and Yahr 1964; Edgington and Dalessio 1970; Aarli et al. 1975; Aarli and Tönder 1980; Sindic et al. 1980).

The present study has been carried out using the complement fixation test. This method allows the use of both soluble and particulate antigens, that not necessarily have to be pure. On the other hand, results obtained are only semiquantitative and anticomplementary activity in the reactants may limit its use. Moreover, it is not a priori known how it is influenced by the non-specific affinity between IgG and CNS.

Several observations indicate that an unspecific binding of IgG is not the basis for the present findings: (a) Positive reactions are found only in some sera, most of them from MS patients, and most sera do not react even undiluted. (b) No correlation was found between IgG level and ABA titre in MS sera (paper V). (c). There was a very dissimilar frequency of positive reactions among MS CSF and non-MS CSF even in tests with a standardized IgG concentration (Ryberg 1976, paper I). (d) There was a virtual lack of correlation between CSF/serum ratios for IgG and ABA (paper IV). (e) Different MS samples show distinct reaction patterns, some being definitively shown to depend on reac-

tions with separate antigens (paper I). In combination with the demonstration of the IgG nature of the serum and CSF reactants (paper II), this latter finding provides firm support for the antigen antibody nature of the reaction.

A conformational change in the Fc part of the IgG molecule, following its specific binding to antigen, has been proposed to increase its affinity to the C1q molecule (Metzger 1978; Boackle et al. 1979). One explanation for the apparent ability of the complement fixation test to discriminate between IgG specifically and non-specifically bound to CNS structures, could be the lack of such conformational change in the latter type of binding. Another explanation would be a less efficient spacing or arrangement of the non-specifically bound IgG molecules. $F(ab')_2$ fragments, often used to assess the antibody nature of an Ig reaction, can naturally not be applied in the Fc dependent complement fixation test.

The association between ABA titre, on one hand, and a more malignant course and disability, on the other (Paper V), raises the important question whether these antibodies are pathogenetic or merely an epiphenomenon of the disease. Several mechanisms, largely attributable to an excessive immunogenic stimulation and/or a defective immunoregulation, can give rise to autoantibodies (Allison 1977). Some of them may be operative in MS. The breakdown and phagocytosis of CNS tissue (Prineas and Raine 1976) and the presence of lymphocytes, plasma cells, and macrophages in the perivascular spaces of MS brain (Prineas 1979) are factors favouring the induction and perpetuation of an autoimmune process. A disordered immunoregulation in MS has now been documented by several groups (Arnason and Antel 1978; Reinherz et al. 1980). Irrespective of the mechanism eliciting ABA in MS, they are potentially pathogenic in one or more of the following ways:

(1) Direct action of antibody. Support for this mechanism is only indirect and derived from experiments with decomplemented serum from animals with EAE. Such serum induces myelin swelling and aberrant myelination in organotypic cultures of mammalian CNS (Raine et al. 1978).

(2) Interaction with complement. In contrast to the complement-dependent in vitro demyelinating and gliotoxic activities in EAE serum (Appel and Bornstein 1964; Berg and Bergstrand 1968),

the corresponding activity in MS serum was found only exceptionally to be immunoglobulin mediated (Grundke-Iqbal and Bornstein 1980). However, IgG from several MS CSF caused demyelination in living tadpoles (Tabira et al. 1977b) and the concept of an IgG mediated activation of complement in the pathogenesis of MS is supported by the observation of IgG and complement firmly bound within MS plaques (Woyciechowska and Brzosko 1977).

Probably unrelated to the demyelinating process, MS sera exhibited complement-dependent, reversible neuroelectric blocking activity in mammalian CNS explants (Bornstein and Crain 1965). In an amphibian in vitro model more specific for demyelinating disease (Cerf und Carels 1966), the activity seemed to be IgG mediated (Schauf and Davis 1978).

Antibodies to gangliosides, which are frequently found in MS sera (Mullin et al. 1980), are known to induce a number of biological effects when injected in the brain of experimental animals, possibly interacting with receptor functions of G_{M1} ganglioside (Rapport et al. 1980). Similarly, antibodies to sulfatide, which were demonstrated in two of 60 MS patients (paper I), have now been found to block opiate mediated effects in rats (Craves et al. 1980). However, it is not known whether or not these in vivo effects are amplified by complement.

(3) Antibody-dependent cell-mediated cytotoxicity (ADCC). This was thought to be the mechanism in experiments where normal human lymphocytes in the presence of MS serum, or MS IgG attacked chicken erythrocytes coated with myelin basic protein (Frick and Stickl 1980), rat brain cultures (Morell 1980), or a rat glioma cell line (Mar 1980). The paucity or virtual absence of lymphocytes in the active edge of the MS plaque (Prineas and Connell 1978) argues against the importance of this mechanism at this site.

(4) Interaction with phagocytic cells. Phagocytic attack on myelin by cells identified as astroglia and microglia has recently been observed in MS plaques (Prineas and Raine 1976; Prineas and Connell 1978). It was suggested (Raine 1977) that macrophages in MS brain attack myelin and oligodendroglia opsonized by specific immunoglobulins, which, however, were not documented. The demonstration of ABA in MS and the finding in MS plaques of macrophages with Fc receptor activity (Nyland et al. 1980) and intracellular, probably ingested, IgG (Esiri 1980) are important

steps in the validation of this model. Further support is provided by the rabbit eye model, where stripping of myelin by a phagocytic process was apparently dependent on anti-CNS antibodies (Brosnan et al. 1977). Conceivably, IgG non-specifically bound to brain membranes by the Fc part (Aarli et al. 1975) would give a less efficient opsonization than ABA specifically bound. The active role of phagocytosis in demyelinating diseases, however, is not unambiguous and macrophages might merely be scavengers of damaged tissue. Such a role is well known in other conditions and was found probable when primary demyelination was induced by injection into the guinea-pig spinal cord of antiserum to myelin (Williams et al. 1980). Antiserum-mediated demyelination preceded phagocytosis in this model.

Macrophages stimulated by T-cell products or involved in antibody mediated phagocytosis exert a number of extracellular effects (Ögmundsdottir and Weir 1980). Proteolytic and other enzymes which are increased in and around MS plaques and in MS CSF (Davison and Cuzner 1977) are thought in part to derive from stimulated phagocytic cells. Such enzymes can digest myelin basic protein in isolated myelin (Cammer et al. 1978) and might play a role in vivo as well (Whitaker 1978); some of them are perhaps related to the non-immunoglobulin in vitro demyelinating factors (Grundke-Iqbal and Bornstein 1980). Thus, ABA might play a role in demyelination also by mechanisms of this kind.

A protective role for ABA? Despite the many pathogenic possibilities for ABA, they have rather been associated with a relative resistance to EAE (Lisak et al. 1970; Lebar and Voisin 1974). The protection against EAE can be transferred by injection of sera containing complement fixing ABA in animals challenged with encephalitogenic preparations (Paterson et al. 1965; Salvin and Liauw 1968), but the protective agents and mechanisms are not known. Anyhow, it is possible that both synergistic and antagonistic effects are exerted in the target tissue by ABA of identical or different properties.

SUMMARY

ABA may be important in the pathogenesis of demyelinating diseases, but their study has been hampered by the unspecific affinity of normal human IgG to components in the CNS. The complement fixation technique, however, appears to discriminate between IgG specifically and non-specifically bound to CNS preparations. Employing this method, the present study for the first time delineated 7 different specificities of ABA in serum and CSF from MS patients. The most prevalent ABA reacted with an antigen in CNS myelin with the properties of an intrinsic membrane protein. Antibodies to two glycolipid haptens, galactosylceramide and sulfatide, the latter not earlier documented in man, were found in low frequency.

Chromatographic experiments and absorptions with bacterial strains with defined immunoglobulin binding characteristics revealed ABA usually to be of IgG class (subclasses 1 and 2).

ABA were not a major constituent of any of the oligoclonal immunoglobulin bands in MS samples separable by thin layer isoelectric focusing.

The distribution of ABA between serum and CSF indicates that they are synthesized on both sides of the blood-brain barrier in proportions that show a great interindividual variation.

A high frequency of ABA in some diseases, viz. SLE, the Guillain-Barré syndrome, and neurosyphilis, cannot yet be ruled out. The present study, however, revealed ABA to be a characteristic feature of MS, being demonstrable in serum and/or CSF in 92 % of patients without disturbing anticomplementary activity. From the absence of ABA in most patients with CNS pathology other than MS, it is clear that the mere destruction of CNS tissue is not a sufficient prerequisite for their production.

No consistent correlations between ABA and clinical events during a longitudinal study were found. Thus, MS relapses are

not caused by a general increase in ABA titres, and conversely the relapses did not cause a boosting of antibrain antibodies. Significant variations in the local plaque environment are, however, not ruled out by the present results.

There was a tendency towards higher CSF ABA titres in younger MS patients and in those with an earlier onset of disease. ABA titres in serum and CSF were both correlated with a more malignant course and disability. Irrespective of the mechanism of induction of ABA in MS - an excessive immunogenic stimulation and/or a defective immunoregulation - they are potentially pathogenic in several ways, e.g. (1) by direct antibody action, (2) by interaction with complement, (3) by antibody-dependent cell-mediated cytotoxicity, and (4) by interaction with phagocytic cells.

From a practical point of view, ABA may prove to be of diagnostic and, possibly, of prognostic value.

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Björn Ryberg

REFERENCES

Aarli, J.A., S.R. Aparicio, C.E. Lumsden and O. Tönder (1975): Binding of normal human IgG to myelin sheaths, glia and neurons. Immunology, 28:171-185.

Aarli, J.A. and O. Tönder (1980): Immunological aspects of neurological diseases. S. Karger, Basel.

Aasjord, P., H. Nyland and S. Mørk (1980): Encephalitis induced in rabbits by staphylococcal lipoteichoic acid. Acta path. microbiol. scand. Sect. C, 88:287-291.

Abramsky, O., R.P. Lisak, D.H. Silberberg and D.E. Pleasure (1977): Antibodies to oligodendroglia in patients with multiple sclerosis. N. Engl. J. Med., 297:1207-1211.

Acheson, E.D. (1972): The epidemiology of multiple sclerosis. In: D. McAlpine, C.E. Lumsden, and E.D. Acheson (Eds.), Multiple sclerosis - A Reappraisal, 2nd edition, Churchill Livingstone, Edinburgh & London, pp. 3-80.

Acheson, E.D. (1977): Epidemiology of multiple sclerosis. Brit. Med. Bull., 33:9-14.

Adams, C.W.M. (1977): Pathology of multiple sclerosis: Progression of the lesion, Brit. Med. Bull., 33:15-20.

Åkesson, B., J. Elovson and G. Arvidson (1970): Initial incorporation into rat liver glycerolipids of intraportally injected ^{3}H glycerol. Biochim. Biophys. Acta, 210:15-27.

Allerand, C.D. and M.D. Yahr (1964): Gamma-globulin affinity for normal human tissue of the central nervous system. Science, 144:1141-1142.

Allison, A.C. (1977): Autoimmune diseases: Concepts of pathogenesis and control: In: N. Talal (Ed.), Autoimmunity. Genetic, immunologic, virologic and clinical aspects. Academic Press, New York, pp. 91-139.

Appel, S.H. and M.B. Bornstein (1964): The application of tissue culture to the study of experimental allergic encephalomyelitis. II. Serum factors responsible for demyelination. J. exp. Med., 119:303-312.

Arnason, B.G.W. and J. Antel (1978): Suppressor cell function in multiple sclerosis. Ann. Immunol. (Inst. Pasteur), 129 C: 159-170.

Batchelor, J.R., A. Compston and W.J. Mc Donald (1978): HLA and multiple sclerosis. Brit. Med. Bull., 34: 279-284.

Berg, O. and H. Bergstrand (1968): Different types of antibodies with a gliotoxic effect in serum from animals with experimental allergic encephalomyelitis. Acta. path. microbiol. scandinav., 73:195-210.

Berg, O., B. Källén (1961): An in vitro gliotoxic effect of serum from patients with multiple sclerosis and some other neurologic conditions. K. fysiogr. Sällsk. Lund Förh., 31:87-96.

Berg, O., B. Källén (1962): Gliotoxic effect of serum from patients with neurological diseases. Lancet, i:1051-1052.

Bloom, B.R. (1980): Immunological changes in multiple sclerosis, Nature (Lond.), 287:275-275.

Boackle, R.J., B.J. Johnson and G.B. Caughman (1979): An IgG primary sequence exposure theory for complement activation using synthetic peptides. Nature (Lond.), 282:742-743.

Bornstein, M.B. (1963): A tissue culture approach to demyelinative disorders. Nat. Cancer Inst. Monog., 11:197-214.

Bornstein, M.B. and S.M. Crain (1965): Functional studies of cultured brain tissues as related to "demyelinative disorders". Science, 148:1242-1244.

Bradbury, M.W.B. and D.F. Cole (1980): The role of the lymphatic system in drainage of cerebrospinal fluid and aqueous humour. J. Physiol., 299:353-365.

Broman, T. (1964): Blood-brain barrier damage in multiple sclerosis. Supravital test-observations. Acta. neurol. scandinav., 40 (Suppl. 10):21-24.

Brosnan, C.F., G.L. Stoner, B.R. Bloom and H.M. Wisniewski (1977): Studies on demyelination by activated lymphocytes in the rabbit eye. II. Antibody-dependent cell-mediated demyelination. J. Immunol., 118:2103-2110.

Brown, W.J. (1978): The capillaries in acute and subacute multiple sclerosis plaques: A morphometric analysis. Neurology (Minneap.), 28(2):84-92.

Bunge, R.P. (1980): Neurological mutants affecting myelination. Nature (Lond.), 286:106-107.

Cammer, W., B.R. Bloom, W.T. Norton and S. Gordon (1978): Degradation of basic protein in myelin by neutral proteases secreted by stimulated macrophages: A possible mechanism of inflammatory demyelination. Proc. Natl. Acad. Sci. (Wash.), 75:1554-1558.

Cerf, J.A. and G. Carels (1966): Multiple sclerosis: Serum factor producing reversible alterations in bioelectric responses. Science, 152:1066-1068.

Cook, D. and P.C. Dowling (1980): Multiple sclerosis and viruses: An overview. Neurology (Minneap.), 30(2): 80-91.

Craves, F.B., B. Zalc, L. Leybin, N. Baumann and H. Loh (1980): Antibodies to cerebroside sulfate inhibit the effects of morphine and β-endorphin. Science, 207:75-76.

Cremer, N.E., K.P. Johnson, G. Fein and W.H. Likosky (1980): Comprehensive viral immunology of multiple sclerosis. II. Analysis of serum and CSF antibodies by standard serologic methods. Arch. Neurol. (Chic.) 37:610-615.

Davison, A.N. and M.L Cuzner (1977): Immunochemistry and biochemistry of myelin. Brit. Med. Bull., 33:60-66.

Delpech, B. and E. Lichtblau (1972): Étude quantitative des immunoglobulines G et de l'albumine du liquide céphalorachidien. Clin. chim. Acta, 37:15-23.

Dupouey, P. (1972): Role of the cerebrosides and a galactodiglyceride in the antigenic cross-reaction between nerve tissue and treponema. J. Immunol., 109:146-153.

Edgington, T.S. and D.J. Dalessio (1970): The assessment by immunofluorescence methods of humoral anti-myelin antibodies in man. J. Immunol., 105:248-255.

Eickhoff, K., W. Kaschka, F. Skvaril, L. Theilkaes and R. Heipertz (1979): Determination of IgG subgroups in cerebrospinal fluid of multiple sclerosis patients and others. Acta neurol. scand., 60:277-282.

Esiri, M.M. (1977): Immunoglobulin-containing cells in multiple-sclerosis plaques. Lancet, 2:478-480.

Esiri, M. (1980): Multiple sclerosis: A quantitative and qualitative study of immunoglobulin-containing cells in the central nervous system. Neuropath. Appl. Neurobiol., 6:9-21.

Fog, T. (1965): The topography of plaques in multiple sclerosis. Acta neurol. scandinav., 41 (Suppl. 15):1-161.

Folchi-Pi, J. (1972): Proteolipids. In: A.N. Davison, P. Mandel and J.G. Morgan (Eds.). Functional and Structural Proteins of the Nervous System. Plenum Press. New York, NY, pp. 171-189.

Frick, E. and L. Scheid-Seydel (1958): Untersuchungen mit J^{131} markiertem γ-Globulin zur Frage der Abstammung der Liquoreiweisskörper. Klin. Wschr. 36:857-863.

Frick, E. and H. Stickl (1980): Antibody-dependent lymphocyte cytotoxity against basic protein of myelin in multiple sclerosis. J. neurol. Sci. 46:187-197.

Fulton, F. and K.R. Dumbell (1949): The serological comparison of strains of influenza virus. J. gen. Microbiol. 3:97-111.

Grundke-Iqbal, J. and M.B. Bornstein (1979): Multiple sclerosis: immunochemical studies on the demyelinating serum factor. Brain Res., 160:489-503.

Grundke-Iqbal, I. and M.B. Bornstein (1980): Multiple sclerosis: Serum gamma globulin and demyelination in organ culture. Neurology (Minneap.) 30:749-754.

Hakomori, S. (1974): Preparation and properties of anti-sulfatide serum, J. Immunol., 112:424-426.

Hakomori, S. and B. Siddiqui (1974): Isolation and characterization of glycosphingolipid from animal cells and their membranes. In: S. Fleischer and L. Packer (Eds), Methods in Enzymology, Vol. XXXII, (Biomembranes, Part B), Academic Press, New York, NY, pp. 345-367.

Hallpike, J.F. (1972): Enzyme and protein changes in myelin breakdown and multiple sclerosis. Progr. Histochem. Cytochem., 3:179-218.

Heimer, R. and F. Levin (1965): Inhibition of complement fixation by rheumatoid sera. Ann. N.Y. Acad. Sci., 124 (II): 879-883.

Hirsch, H.E. and M.E. Parks (1976): Serological reactions against glycolipid-sensitised liposomes in multiple sclerosis. Nature (Lond.), 264:785-787.

Hogan, E.L. (1977): Animal models of genetic disorders of myelin. In: P. Morell (Ed.), Myelin, Plenum Press, New York and London, pp. 489-520.

Jaque, C.M., O.S. Jørgensen, N.A. Baumann and E. Bock (1976): Brain-specific antigens in the quaking mouse during ontogeny. J. Neurochem. 27:905-909.

Kabat, E.A., D.H. Moore and H. Landow (1942): An electrophoretic study of the protein components in cerebrospinal fluid and their relationship to the serum proteins. J. clin. Invest., 21:571-577.

Källén, B., B. Löw and O. Nilsson (1975): Mixed leukocyte reaction and HL-A specificity at multiple sclerosis. Acta neurol. scand. 51:184-192.

Karlsson, K-A., B.E. Samuelsson and G.O. Steen (1973): The sphingolipid composition of bovine kidney cortex, medulla and papilla. Biochim. Biophys. Acta, 316:317-335.

Kates, M. (1972): Separation of lipid mixtures. In: T.S.Work and E. Work (Eds.), Techniques of Lipidology - Isolation, Analysis and Identification of Lipids, North-Holland Publishing Company, Amsterdam, pp. 428-446.

Kennedy, P.G.E. and Lisak (1981): Do patients with demyelinating diseases have antibodies against human glial cells in their sera? J. Neurol. Neurosurg. Psych., 44:164-167.

Kronvall, G., A. Simmons, E.B. Myhre and S. Jonsson (1979): Specific absorption of human serum albumin, immunoglobulin A,and immunoglobulin G with selected strains of group A and G streptococci. Infect. Immunity, 25:1-10.

Lamoreux, G. and A.G. Borduas (1966): Immune studies in multiple sclerosis. Clin. exp. Immunol., 1:363-376.

Laurell, A-B and H. Link (1972): Complement-fixing antibrain antibodies in multiple sclerosis. Acta neurol. scand., 48:461-466.

Lebar, R. and G.A. Voisin (1974): Studies on auto-immune encephalomyelitis in the guinea pig. Int. Arch. Allergy 46: 82-103.

Link, H. (1967): Immunoglobulin G and low molecular weight proteins in human cerebrospinal fluid. Acta neurol. scand., 43 (Suppl. 28):1-136.

Link, H. (1972): Oligoclonal immunoglobulin G in multiple sclerosis brains. J. neurol. Sci., 16:103-114.

Lisak, R.P. (1980): Multiple sclerosis: Evidence for immunopathogenesis. Neurology (Minneap.) 20 (2):99-105.

Lisak, R.P., G.A. Falk, R.G. Heinze, M.W. Kies and E.C. Alvord (1970): Dissociation of antibody production from disease suppression in the inhibition of allergic encephalomyelitis by myelin basic protein. J. Immunol., 104:1435-1446.

Lisak, R.P., B. Zwiman and M. Norman (1975): Antimyelin antibodies in neurologic diseases. Immunofluorescent demonstration. Arch. Neurol. (Chic.), 32:163-167.

Lowenthal, A. (1964): Agar gel electrophoresis in neurology. Elsevier Publishing Company, Amsterdam.

Lumsden, C.E. (1971): The immunogenesis of the multiple sclerosis plaque. Brain Res., 28:365-390.

Lumsden, C.E. (1972): The clinical immunology of multiple sclerosis. In: D. McAlpine, C.E. Lumsden and E.D. Acheson (Eds.), Multiple Sclerosis - A Reappraisal, 2nd edition, Churchill Livingstone Edinburgh, London, pp. 512-621.

Mancini, G., A.O. Carbonara and J.F. Heremans (1965): Immunochemical quantification of antigens by single radial immunodiffusion. Immunochemistry, 2:235-254.

Mar, P. (1980): Antibody-dependent cellular cytotoxicity in multiple sclerosis. J. neurol. Sci., 47;285-303.

Marshall, J. (1955): Spastic paraplegia of middle age-A clinicopathological study. Lancet, 1:643-646.

Mattson, D.H., R.P. Roos and B.G.W. Arnason (1980): Isoelectric focusing of IgG eluted from multiple sclerosis and subacute sclerosing panencephalitis brains. Nature (Lond.), 287:335-337.

McDonald, W.J. and A.M. Halliday (1977): Diagnosis and classification of multiple sclerosis. Brit. Med. Bull., 33:4-8.

Melnick, S.C. (1963): Thirty-eight cases of the Guillain-Barré syndrome: An immunological study. Br. med. J., 1:368-373.

Metzger, H. (1978): The effect of antigen on antibodies: Recent studies. In: R.A. Reisfeld and F.P. Inman (Eds.), Contemporary topics in molecular immunology, Vol 2, Plenum Press, New York & London, pp. 119-152.

Mies, H.-J. (1953): Einegung von Liquor cerebrospinalis als Vorbereitung zur Papierelectro-Phorese. Klin. Wschr., 31:159-161.

Morell, R.M. (1980): Immunoglobulin (Ig) from multiple sclerosis (MS) lymphocyte surface confers cytotoxic potential on normal mononuclear cells. Neurology (Minneap.), 30:398.

Müller, R. (1949): Studies on multiple sclerosis. Acta med. scand 133 (Suppl. 222):1-214.

Mullin, B.R., A.J. Montanaro, J.D. Reid and N. Nishimura (1980): Interaction of multiple sclerosis serum with liposomes containing ganglioside G_{M1}. Ann Neurol. 7:587-590.

Nordal, H.J., B. Vandvik and E. Norrby (1978): Demonstration of electrophoretically restricted virus-specific antibodies in serum and cerebrospinal fluid by imprint electroimmunofixation. Scand. J. Immunol., 7:381-388.

Norrby, E. (1978): Viral antibodies in multiple sclerosis. Progr. med. Virol., 24:1-39.

Norrby, E., H. Link, J.-E. Olsson, M. Panelius, A. Salmi and B. Vandvik (1974):Comparison of antibodies against different viruses in cerebrospinal fluid and serum samples from patients with multiple sclerosis. Infect. Immun., 10:688-694.

Norrby, E. and B. Vandvik (1975): Relationship between measles virus-specific antibody activities and oligoclonal IgG in the central nervous system of patients with subacute sclerosing panencephalitis and multiple sclerosis. Med. Microbiol. Immunol. (Berl.), 162:63-72.

Nyland, H. and J.A. Aarli (1978): Guillain-Barré syndrome: demonstration of antibodies to peripheral nerve tissue. Acta neurol. scand., 58:35-43.

Nyland, H., R. Matre and S. Mørk (1980): Fc receptors on microglial lipophages in multiple sclerosis. N. Engl. J. Med., 302:120-121.

Ögmundsdottir, H.M. and D.M. Weir (1980): Mechanisms of macrophage activation. Clin. exp. Immunol., 40:223-234.

Olsson, J.-E., H. Link and R. Müller (1976): Immunoglobulin abnormalities in multiple sclerosis. Relation to clinical parameters: disability, duration and age of onset. J. neurol. Sci., 27:233-245.

Palmer, D.L., B.J. Minard and L.P. Cawley (1976): IgG subgroups in cerebrospinal fluid in multiple sclerosis., New Engl. J. Med. 294:447-448.

Panitsch, H.S., C.J. Hooper and K.P. Johnson (1980): CSF antibody to myelin basic protein. Measurement in patients with multiple sclerosis and subacute sclerosing panencephalitis. Arch. Neurol. (Chic.), 37:206-209.

Parish, C.R., D.C. Jackson and J.F.C. McKenzie (1976): Low-molecular-weight Ia antigens in normal mouse serum. III. Isolation and partial chemical characterization. Immunogenetics, 3:455-463.

Paterson, P.Y., E.D. Day and C.C. Whitacre (1981): Neuroimmunologic diseases: Effector cell responses and immunoregulatory mechanisms. Immunological Rev., 55:89-120.

Paterson, P.Y., A.F. Jacobs and E.M. Coia (1965): Complement-fixing antibrain antibodies and allergic encephalomyelitis. II. Further studies concerning their protective role. Ann. N.Y. Acad. Sci., 124(I):292-298.

Poser, S., J. Herrman-Gremmels, J. Wikström and W. Poser (1978): Clinical features of the spinal form of multiple sclerosis. Acta neurol. scandinav., 57:151-157.

Poskanzer, D.C., L.B. Prenney, J.L. Sheridan, and J.Y. Kondy (1980): Multiple sclerosis in the Orkney and Shetland islands. I: Epidemiology, clinical factors, and methodology. J. Epidemiol. Comm. Health., 34:229-239.

Prineas, J. (1975): Pathology of the early lesions in multiple sclerosis. Hum. Pathol., 6:531-554.

Prineas, J.W. (1979): Multiple sclerosis: Presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. Science 203:1123-1125.

Prineas, J.W. and F. Connell(1978): The fine structure of chronically active multiple sclerosis plaques. Neurology (Minneap.) 28(2):68-75.

Prineas, J.W. and C.S. Raine (1976): Electron microscopy and immunoperoxidase studies of early multiple sclerosis lesions. Neurology (Minneap.) 26(2):29-32.

Raine, C.S. (1977): The etiology and pathogenesis of multiple sclerosis: Recent developments. Pathobiol. Ann. 7:347-384.

Raine, C.S., M. Diaz, M. Pakingan and M.B. Bornstein (1978): Antiserum-induced dissociation of myelinogenesis in vitro. An ultrastructural study. Lab. Invest. 38:397-403.

Rapport, M.M. and L. Graf (1967): Preparation and testing of lipids for immunological study. In: C.A. Williams and M.W. Chase (Eds.), Methods in Immunology and Immunochemistry, Vol. 1, Academic Press, New York, NY, pp. 187-196.

Rapport, M.M., S.E. Karpiak and S.P. Mahadik (1980): Perturbation of CNS functions by antibodies to gangliosides. Speculations on biological roles of ganglioside receptors. In: L. Svennerholm, P. Mandel, H. Dreyfus and P.-F. Urban (Eds.), Symposium on structure and function of gangliosides. Plenum Press, New York & London, pp. 335-338.

Rastogi, S.C., J. Clausen and T. Fog (1978): Multiple sclerosis specific antigens in MS brains. Acta neurol. scandinav. 57:438-442.

Reinherz, E.L., H.L. Weiner, S.L. Hansen, J.A. Cohen, J.A. Distaso and S.F. Schlossman (1980): Loss of suppressor T cells in active multiple sclerosis. N. Engl. J. Med., 303: 125-129.

Riski, H., S. Pyrhönen, O. Wager and K. Penttinen (1977): Lack of measurable complement fixing antibodies against virus antigens. Acta Path. Microbiol. Scand., Sect. B, 85:167-173.

Ryberg, B. (1975): Complement-fixing antibrain antibodies in multiple sclerosis. Presentation at The 21st scandinavian congress of neurology, Stockholm 1975.

Ryberg, B. (1976): Complement-fixing antibrain antibodies in multiple sclerosis. Acta neurol. scand. 54:1-12.

Ryberg, B. (1978): Multiple specificities of antibrain antibodies in multiple sclerosis and chronic myelopathy. J. neurol. Sci., 38:357-382.

Ryberg, B. (1980): Intrathecal and extrathecal production of antibrain antibodies in multiple sclerosis. J. neurol. Sci., 48:1-8.

Ryberg, B. (1981): A longitudinal study of antibrain antibodies in multiple sclerosis. Submitted for publication.

Ryberg, B. (1981): Antibrain antibodies in multiple sclerosis. Relation to clinical variables. Submitted for publication.

Ryberg, B. and G. Kronvall (1981): Immunoglobulin characterization by bacterial absorption of antibrain antibodies in multiple sclerosis. Eur. Neurol. In press.

Sachs, H. and G. Steiner (1934): Serologische Untersuchungen bei multipler Sklerose. Klin. Wschr., 13:1714-1717.

Sandberg-Wollheim, M. (1974): Immunoglobulin synthesis in vitro by cerebrospinal fluid cells in patients with multiple sclerosis. Scand. J. Immunol., 3:717-730.

Salvin, S.B. and H.L. Liauw (1968): Immunologic unresponsiveness, immunity and enhancement in experimental allergic encephalomyelitis. J. Immunol., 101:33-42.

Sheremata, W., D.D. Wood, A.A. Moscarello and J.B.R. Cosgrove (1978): Sensitization to myelin basic protein in attacks of multiple sclerosis. A preliminary report. J. neurol. Sci., 36:165-170.

Schauf, C.L. and F.A. Davis (1978): The occurrence, specificity, and role of neuroelectric blocking factors in multiple sclerosis. Neurology (Minneap.), 28 (2):34-39.

Schochet, A.L., H.L. Weiner, J. Walker, K. McIntosh and P.F. Kohler (1977): Lymphocytotoxic antibodies in multiple sclerosis. Clin. Immunol. Immunopath., 7:15-23.

Schumacher, G.A., G. Beebe, R.F. Kibler, L.T. Kurland, J.F. Kurtzke, F. McDowell, B. Nagler, W.A. Sibley, W.W. Tourtellotte and T. Willmon (1965): Problems of experimental trials of therapy in multiple sclerosis - Report of the panel on the evaluation of experimental trials of therapy in multiple sclerosis, Ann. N.Y. Acad. Sci., 122:552-561.

Shorr, J., B. Roström and H. Link (1981): Antibodies to viral and non-viral antigens in subacute sclerosing panencephalitis and multiple sclerosis demonstrated by thin-layer polyacrylamide gel isoelectric focusing, antigen immunofixation and autoradiography. J. neurol. Sci., 49:99-108.

Sindic, C.J.M., C.L. Cambiaso, P.L. Masson and E.C. Laterre (1980): The binding of myelin basic protein to the Fc region of aggregated IgG and to immune complexes. Clin. Exp. Immunol., 41:1-7.

Sörnäs, R. (1967): A new method for cytological examination of cerebrospinal fluid. J. Neurol. Neurosurg. Psychiatry, 30:568-577.

Spiegelberg, H.L. (1974): Biological activities of immunoglobulins of different classes and subclasses. Adv. Immunol., 19:259-293.

Tabira, T., H. De F. Webster and S.H. Wray (1977 a): In vivo test for myelinotoxicity of cerebrospinal fluid. Brain Res., 120:103-112.

Tabira, T., F.J. Wolfgram, H. De F. Webster, S.H. Wray and D.E. McFarlin (1977b): Myelinotoxicity of CSF fractions from multiple sclerosis patients tested in an in vivo model. Neurology (Minneap.), 27:374.

Tibbling, G., H. Link and S. Öhman (1977): Principles of albumin and IgG analysis in neurological disorders. I. Establishment of reference values. Scand. J. clin. Lab. Invest., 37:385-390.

Tourtellotte, W.W. (1975): What is multiple sclerosis? Laboratory criteria for diagnosis. In: A.N. Davison, J.H. Humphrey, A.L. Liversedge, W.J. McDonald and J.S. Porterfield (Eds.). Multiple sclerosis Research, Her Majesty's Stationary Office, London, pp. 9-26.

Traugott, V., D.S. Snyder and C.S. Raine (1979): Oligodendrocyte staining by multiple sclerosis serum is nonspecific. Ann. Neurol., 6:13-20.

Vandvik, B., J.B. Natvig and D. Wiger (1976): IgG_1 subclass restriction of oligoclonal IgG from cerebrospinal fluids and brain extracts in patients with multiple sclerosis and subacute encephalitides. Scand. J. Immunol., 5:427-436.

Vandvik, B., E. Norrby and H.J. Nordal (1979): Optic neuritis: Local synthesis in the central nervous system of oligoclonal antibodies to measles, mumps, rubella and herpes simplex viruses. Acta neurol. scand., 60:204-213.

Whitaker, J.N. (1978): The distribution of myelin basic protein in central nervous system lesions of multiple sclerosis and acute experimental allergic encephalomyelitis. Ann. Neurol., 3:291-298.

Wieme, R.J. (1959): An improved technique of agar gel electrophoresis on microscopic slides. Clin. chim. Acta, 4:317-321.

Williams, A., R. Eldrigde, H. McFarland, S. Houff, H. Krebs and D. McFarlin (1980): Multiple sclerosis in twins. Neurology (Minneap.) 30:1139-1147.

Williams, R.M., S. Krakowka and A. Koestner (1980): In vivo demyelination by antimyelin antibodies. Acta Neuropathol., (Berl.), 50:1-8.

Woyciechowska, J.L. and W.J. Brzosko (1977): Immunofluorescence study of brain plaques from two patients with multiple sclerosis. Neurology (Minneap.) 27:620-622.

Yokoyama, M., E.G. Trams and R.O. Brady (1962): Sphingolipid antibodies in sera of animals and patients with central nervous system lesions. Proc. Soc. exp. Biol. Med. (N.Y.), 111:350-352.

APPENDIX

Papers I-VI

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MULTIPLE SPECIFICITIES OF ANTIBRAIN ANTIBODIES IN MULTIPLE SCLEROSIS AND CHRONIC MYELOPATHY

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SUMMARY

The presence of complement-fixing antibodies against brain antigens was tested in paired serum and cerebrospinal fluid (CSF) samples from 60 multiple sclerosis (MS) patients, 15 patients with chronic myelopathy of undetermined cause (CM) and 60 control patients. Six MS sera, 34 MS CSF, 4 CM sera, 3 CM CSF, 4 control sera and 1 control CSF gave positive reactions either with a lipid extract or a saline extract of normal human brain. The proportion of anticomplementary CSF was significantly higher in the MS group than in the control group (15% vs 0%, $P < 0.01$). The reactivity of a large number of individual positive samples was further investigated. Seven antibody specificities were discerned in the MS samples. Most samples reacted with nonlipid antigens, the dominating being a heat-labile, nonlipid component associated with CNS myelin. Antibodies to cerebroside and sulfatide were detected in a few patients. A number of samples reacted with cholesterol in combination with a variety of lipids. Positive samples from the CM patients exhibited a similar heterogeneity. In the control group positive reactions were seen in one patient with systemic lupus erythematosus (SLE), two patients with rheumatoid arthritis (RA), and one with a spinal meningioma. The reaction patterns of these patients were different from those commonly seen in MS patients. The complement-fixing antibrain antibodies in MS CSF are usually of IgG class (Ryberg 1976). This applies also to the positive MS sera in this study. The distribution of the antibodies between serum and CSF indicated, in several cases, an intrathecal synthesis. All of a number of human brains, including

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Abbreviations: CM = chronic myelopathy; GMH = grey matter homogenate; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; TLA = "total lipid antigen"; TLC = thin layer chromatography; WMH = white matter homogenate.

one MS brain, contained all 6 antigens (haptens) reactive in saline extracts. Antibodies to tissues outside the CNS were rarely detected in MS patients. The varied humoral autoimmune response in MS might reflect a heterogeneity in the MS patients, the disease itself or its causative agent.

INTRODUCTION

Recent work has demonstrated the frequent occurrence of complement fixing antibodies against brain antigen in MS CSF in contrast to CSF from other patient categories (Laurell and Link 1972; Ryberg 1976). The antibodies were found to be of IgG class. They also appeared to be specific to CNS antigens and were only exceptionally found in MS serum diluted to the same IgG concentration (Ryberg 1976). In these studies crude brain extracts in aqueous media were used. Experiments with positive serum and CSF from one MS patient showed the antibrain antibody to be specific to sulfatide, a glycolipid not earlier documented as an autoantigen in man (Ryberg, unpublished results). On the other hand, most CSF samples from MS patients seemed to react with other brain antigens.

The purpose of the present work was: (1) to study the occurrence of antibodies in serum and CSF from patients with MS, CM and other neurological diseases to antigens in saline extracts and lipid extracts of brain, (2) to study the specificities of the antibodies and to make a preliminary characterization of the antigens involved, (3) to determine the site of production of the antibodies.

MATERIALS AND METHODS

Paired samples of serum and CSF were obtained from patients belonging to the following groups:

Group 1. Twenty-three male and 37 female unselected MS patients diagnosed according to the clinical criteria given by Müller (1949) and Schumacher, Beebe, Kibler, Kurland, Kurtzke, McDowell, Nagler, Sibley, Tourtellotte and Willmon (1965). However, only patients with two or more exacerbations, separated by a remission of one month or more, were included. No signs of other diseases were present in any of the patients. Agar gel electrophoresis of the actual or a previous CSF sample had shown two or more M-components in the gamma region in 57 of the cases.

Group 2. Fifteen patients with chronic myelopathy of undetermined cause. They had a slowly progressive spastic paraparesis and sometimes other signs of amyelopathy for which no specific cause could be found (Marshall 1955). Oxygen myelography and/or electromanometric pressure recording (Gilland 1966) gave normal results in all these patients. In 13 the level of vitamin B 12 and folic acid was determined and found normal. In 14 serological tests for syphilis were performed and found negative. Agar gel electrophoresis of the CSF revealed 2 or more M-components in the gamma region in 8 of them.

Group 3. Seven patients without evidence of neurological disease and 53 with clinical evidence of pathological affection of the nervous system. Among them were

13 with rhizopathies, 10 with compressive myelopathies (3 of them also suffering from rheumatoid arthritis, RA), 5 with cerebral infarction (the samples being taken respectively 2, 5, 5, 12 and 59 days from the outset of disease), 5 with amyotrophic lateral sclerosis, 4 with infection of the CNS, 4 with the Guillain–Barré syndrome, 3 with epilepsy (one of them having neurofibromatosis), 2 with subarachnoid bleeding (one of them having an intracerebral arteriovenous malformation), 2 with cerebral palsy, 1 with alcoholic cerebellar degeneration, 1 convalescent after severe cerebral concussion, 1 with polyneuropathy, 1 with granulomatous arteritis of the CNS and 1 with SLE affecting the CNS. Except for the SLE-patient and two of the patients with RA, none were treated with ACTH or corticosteroids at the time of sampling.

After cell counting and centrifugation, the CSF specimen was heated to 56 °C for 30 min simultaneously with the serum sample, and concentrated about 100 times by ultrafiltration in a collodion bag (Sartorius, Membranfilter GmbH, Göttingen, West-Germany) as described by Mies (1953). A few millilitres of the unconcentrated, unheated CSF was kept for future controls. The total protein and IgG concentrations were determined in the serum and CSF specimens. All samples were stored at —20 °C. Before testing in the complement fixation test, the serum and CSF samples were diluted with veronal buffer to contain 1000 mg IgG/l. In some instances more diluted serum and CSF were also tested.

Cholesterol of high purity was obtained from BDH Chemicals Ltd, Poole, England, from Supelco Inc., Bellefonte, PA, U.S.A. and from Serva Feinbiochemica, Heidelberg, West-Germany. Phosphatidyl choline from egg yolk was kindly supplied by Dr. Björn Åkesson, Department of Medical Chemistry, University of Lund. L-α-Phosphatidyl choline from soybean, synthetic L-α-phosphatidyl choline, dimyristoyl and synthetic L-α-phosphatidyl choline, dipalmitoyl were purchased from Sigma, Saint Louis, MO, U.S.A. Sphingomyelin from sheep erythrocytes and glucosylceramide from Gaucher's spleen were from Supelco. These substances were found to be free of impurities on TLC with chloroform–methanol–water (65:25:4, v/v/v) as running phase. Low purity egg lecithin was obtained from Sigma.

Quantitation of IgG and IgM was performed with single radial immunodiffusion (Mancini, Vaerman, Carbonara and Heremans 1964) modified as described by Link, Zetterwall and Blennow (1972). Specific rabbit antisera were purchased from the Central Laboratory of the Netherlands Red Cross.

Electrophoresis in agar gel was performed according to the procedure of Wieme (1959). Concentrated CSF was applied in an amount corresponding to 5 μg IgG/1 mm of the application through (Link 1973).

Volumes of 0.25 ml of serum, previously heated to 56 °C for 30 min, were separated at 4°C on a 0.9 × 56 cm bed of Sephadex G-200 Superfine in 0.9% NaCl with 0.02% sodium azide. Fractions of about 0.5 ml were collected every hour. They were examined for optical extinction at 280 nm, assayed for IgG and IgM content and were tested with appropriate antigen in the complement fixation test. A partial separation of IgG and IgM was obtained (Fig. 1).

Tissue samples without macroscopic disease involvement were obtained at autopsy 12–36 hr after death from patients without disseminated neoplasm or infection

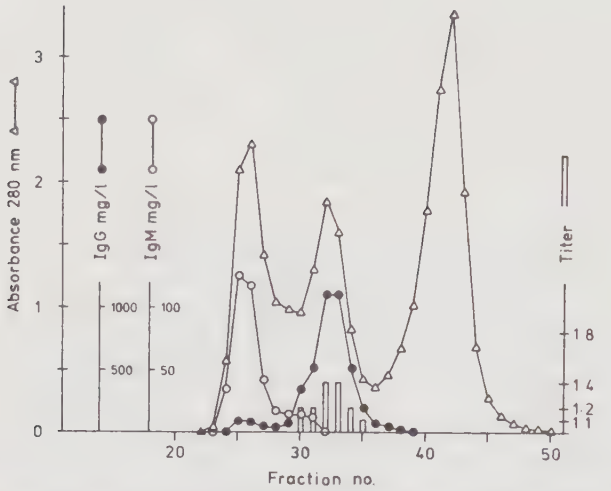

Fig. 1. Reaction with WMH of fractions of serum 76 after gel filtration on Sephadex G 200. The positive reactions are restricted to the IgG-rich fractions.

and without systemic disease. Tissue was also obtained from one patient with a clinical and post-mortem diagnosis of MS and gastric carcinoma. Bovine and rat brains were obtained shortly after the animals were killed. The tissue samples were stored at —70 °C until further prepared.

Brain extract for the screening test was obtained by treating brain tissue (grey and white matter) at top speed in a Bühler homogenizer for 10 min at close to 0 °C in 0.9 % NaCl with 0.02 % sodium azide in a ratio of 1:6 (w/v). The homogenate was centrifuged at 400 × g_{av} for 10 min, and fresh supernatant was used. The supernatant contained approximately 5 mg/ml of dry material after dialysis against distilled water and lyophilization. For the determination of tissue and organ specificity and temperature resistance a different method was employed. Tissue samples were disrupted in cold saline in a ratio of 1:50 (w/v) by treatment for 30 sec with a Polytron at top speed. The homogenates were used fresh and without centrifugation. The tissue extracts and homogenates were generally free from disturbing anticomplementary activity, as was also the MS brain. Before testing, a whole brain homogenate in saline (1:50, w/v) was partitioned into tubes kept at 4°, 20°, 30°, 40°, 50°, 60°, 70° and 80°C respectively, for 10 min to determine the heat resistance of the antigens. Five ml of saline brain extract was separated by gel filtration at 4 °C on a 1.4 × 76 cm bed of Sepharose 4B in 0.9 % NaCl with 0.02 % sodium azide. The optical extinction of the collected fractions was measured at 280 nm.

Myelin was prepared from fresh human white matter by a modification (Hallpike 1972) of the method of Laatsch, Kies, Gordon and Alvord (1962). The water-shocked myelin was stored at —70 °C until tested. Some of it was lyophilized after repeated washings in distilled water. Before testing it was dispersed in 0.9 % NaCl by Polytron treatment. The myelin was essentially pure as determined by electron microscopy. For this examination it was fixed according to Karnovsky (1965) and postfixed in 1 % OsO_4. After dehydration contrast was obtained with 1 % phosphotungstatic acid, 0.5 %

uranylacetate in absolute ethanol. The preparations were embedded in Vestopal W and ultrathin sections were cut on a LKB-ultrotome III. The sections were stained according to Venable and Coggshall (1965) and examined with a Philips 300 electron microscope.

The method of Folch-Pi (1972) was used to obtain lipid extracts. A lipid extract from human brain was subjected to mild alkaline hydrolysis and partitioned as described by Karlsson, Samuelsson and Steen (1973) in order to remove the glycerol ester lipids.

Lipid samples to be separated for analytical or preparatory purposes were dissolved in chloroform–methanol (2:1, v/v) and applied to 20 × 20 cm glass plates coated with a 0.5 mm layer of Silica gel H, type 60 (Merck, Darmstadt, West-Germany) that had been activated at 110 °C for 1 hr and stored in a desiccator until used. The plates were developed in chloroform–methanol–water (65:25:4, v/v/v) (Kates 1972). A separate lane containing standard substances was included when judged useful. After drying, the analytical plates were sprayed with a sulphuric acid–dichromate spray and developed at 180 °C. The preparatory plates were sprayed with an 0.2% solution of 2,7-dichlorofluoresceine in ethanol and inspected and marked under ultraviolet light (Kates 1972). Fractions of interest were scraped off and eluted 3 times with 4 ml chloroform–methanol–acetic acid–water (50:39:1:10, v/v/v/v). The combined extracts were shaken with 4 ml 4 M ammonia. After partition the lower phase was shaken with 4 ml methanol–water (1:1, v/v) and again partitioned (Åkesson, Elovson and Arvidson 1970). The lower phase was evaporated under a stream of nitrogen and the residue was redissolved in chloroform–methanol (2:1, v/v).

Total glycolipids were obtained from 30 g brain tissue by ethanol extraction according to Hakomori and Siddiqui (1974). After extraction twice with aceton and twice with diethyl ether, the lipid pellet was dried and redissolved in chloroform–methanol (2:1, v/v), filtered and evaporated. The residue was subjected to mild alkaline hydrolysis and partitioned (Karlsson et al. 1973). The dried residue weighed 267 mg. It was dissolved in 50 ml chloroform + 2 ml methanol and applied to a chromatographic column loaded with 10 g heat-activated silicic acid. The column was eluted by stepwise addition of chloroform, chloroform–methanol (9:1, v/v), chloroform–methanol (4:1, v/v) and methanol. The fractions containing cerebroside and sulfatide were collected and these substances were further purified by TLC with chloroform–methanol–acetic acid–water (65:25:4:4, v/v/v/v) and chloroform–methanol–4 M ammonia (65:25:4, v/v/v) as running media. Subfractions of cerebroside and sulfatide representing subtypes with normal and hydroxy fatty acid residue were isolated. Conversion of sulfatide to cerebroside by mild acid hydrolysis was performed as described by Karlsson, Samuelsson and Steen (1971). When stored, the lipids were dissolved in chloroform–methanol (2:1, v/v) and kept at —20 °C.

Unless otherwise stated, the saline tissue extracts and homogenates were tested fresh and undiluted. Only preparations with very low anticomplementary activity were used. A cholesterol-fortified "total lipid antigen" (TLA) from different organs was obtained as follows: An amount of lipid extract corresponding to 1 mg dry substance was evaporated under nitrogen and 300 μg cholesterol was added. This material

was dissolved in 0.1 ml ethanol and 0.9 ml of 0.9 % NaCl was added. Before testing this antigen was diluted 1:8 in 0.9 % NaCl. The addition of cholesterol in approximately the described proportions was found greatly to increase the activity of the brain antigen in some instances without making it anticomplementary. With a higher proportion of cholesterol anticomplementarity reached a disturbing level. Working test antigens from brain lipid samples treated in different ways and TLC fractions thereof were obtained by adding egg lecithin and cholesterol on an empirical basis. These auxiliary lipids were generally employed in the weight proportions 1:1 and 1:3. The dried lipids were dissolved together in 0.1 ml ethanol and 0.9 ml of 0.9 % NaCl was added (Rapport 1967). Some of the antigenic mixtures had to be diluted to reduce the anticomplementary activity.

Serum and CSF samples at a concentration several times above the lower limit of antibody detection were absorbed for 1 hr at 37 °C and overnight at 4 °C with tissue homogenates or myelin suspensions in saline. The supernatants were collected after centrifugation at 2000 $\times$ g_{max} for 1 hr.

The complement fixation test was performed according to the micromethod of Fulton and Dumbell (1949). Five 50 % haemolysis units (MHD_{50}) of complement were employed and a photometrically standardized 0.2 % suspension of sheep erythrocytes sensitized with 6 MHD_{50} of amboceptor served as haemolytic system. The reaction was performed at 4 °C overnight and, after the addition of sensitized sheep erythrocytes, the plates were incubated at 37 °C for 2 hr before reading. Fifty per cent or less haemolysis, visually estimated, was recorded as a positive reaction. Controls for anticomplementary activity were performed with antigen, serum and CSF at twice the concentration used in the test.

The χ^2-test was used to test the significance of varying frequencies in the different groups.

RESULTS

Screening tests

All serum and CSF samples from the 3 patient groups were examined at an IgG concentration of 1000 mg/l in the complement fixation test against saline brain extract

TABLE 1

AGE DISTRIBUTION

Age (years)	Group 1 (n = 60)	Group 2 (n = 15)	Group 3 (n = 60)
11–20	2	1	5
21–30	10	1	4
31–40	24	2	11
41–50	19	2	14
51–60	5	3	12
61–70		4	8
71–80		2	5
81–90			1

TABLE 2

THE DISTRIBUTION OF ANTICOMPLEMENTARY AND POSITIVE SAMPLES AMONG THE TEST GROUPS AND THE ANTIGENS

	Anticomplementary		Saline brain extract		TLA from brain		Either antigen	
	serum	CSF	serum	CSF	serum	CSF	serum	CSF
Group 1 (n = 60)	0 (0%)	9 (15%)	5 (8%)	32 (53%)	2 (3%)	4 (7%)	6 (10%)	34 (57%)
Group 2 (n = 15)	0 (0%)	1 (7%)	2 (13%)	2 (13%)	2 (13%)	2 (13%)	4 (27%)	3 (20%)
Group 3 (n = 60)	1 (2%)	0 (0%)	2 (3%)	1 (2%)	3 (5%)	1 (2%)	4 (7%)	1 (2%)

and TLA from brain. Tables 2 and 3 give the results of this screening. A number of samples were anticomplementary* and could not be evaluated concerning their reaction with the antigens. The proportion of anticomplementary CSF samples in the first group was significantly higher ($\chi^2 = 9.73$, d.f.1. $P < 0.01$) than in the third group. The proportion of CSF in Group 1 giving positive reaction with saline brain extract was significantly higher than in Group 2 ($\chi^2 = 7.75$, d.f.1. $P < 0.01$) and Group 3 ($\chi^2 = 40.16$,d.f.1. $P < 0.0001$). If the anticomplementary samples are excluded from the calculations the proportion of MS CSF reactive with saline brain extract is 63%, with TLA 8%, and with either antigen 67%. All sera and several CSF reactive with saline brain extract were further characterized immunologically. Although a number of samples from the patients were usually available the material was limited and did not allow all tests to be performed in all patients.

Organ specificity

An extensive test for organ specificity was made with sera. Homogenates in saline (1:50, w/v) of the following human tissues were tested: cerebral hemisphere, cerebellum, pons, medulla oblongata, semilunar ganglion, peripheral (femoral) nerve, neurohypophysis (as an example of nonmyelinated CNS tissue), epiphysis, plexus chorioideus, dura mater, adipose tissue, nucleus pulposus, skin, striated muscle, myocardium, arterial wall, lung, thymus, spleen, lymphatic gland, oesophagus, gastric mucosa (fundus, corpus and anthrum), pylorus, duodenum, small intestine, colon, liver, pancreas, gall bladder, adenohypophysis, thyroid, adrenal gland (cortex and medulla), testis, epididymis, prostate, ovary, uterus, placenta, kidney (cortex and medulla) and urinary bladder. Table 4 gives the results of this test; it also gives the findings with the serum with antibodies to sulfatide (here designated A13) that was found during preliminary studies. For CSF the test for organ specificity included homogenates of brain, semilunar ganglion, peripheral nerve, neurohypophysis, kidney, and in some cases myocardium and spleen. Table 5 lists the results.

* The term anticomplementary is used to denote the property whereby a sample (antibody or antigen source) spontaneously binds or inactivates complement. It may, for example, be caused by certain immunoglobulins in the form of immune complexes or non-specific aggregates and certain lipids in purified form.

TABLE 3

THE REACTION OF SERUM AND CSF IN PATIENTS HAVING ANY KIND OF POSITIVE REACTION IN THE SCREEN TEST AGAINST BRAIN EXTRACTS

Code number of the patient	Diagnosis	Saline brain extract		TLA from brain	
		serum	CSF	serum	CSF
Group 1					
8	MS	—	+	—	—
12	MS	—	+	—	—
14	MS	—	+	—	+
16	MS	—	—	—	+
17	MS	—	+	—	—
27	MS	—	+	—	—
28	MS	—	+	—	—
33	MS	—	+	—	—
38	MS	—	+	—	—
44	MS	—	+	—	—
46	MS	—	+	—	+
47	MS	—	+	—	—
55	MS	+	+	—	—
56	MS	—	+	—	—
63	MS	—	+	—	—
64	MS	—	+	+	—
65	MS	—	+	—	—
67	MS	—	+	—	—
74	MS	—	+	—	—
75	MS	—	+	—	—
76	MS	+	+	+	—
77	MS	—	—	—	+
78	MS	—	+	—	—
82	MS	+	—	—	—
85	MS	—	+	—	—
90	MS	—	+	—	—
96	MS	—	+	—	—
100	MS	+	+	—	—
101	MS	+	ac[a]	—	ac[a]
106	MS	—	+	—	—
108	MS	—	+	—	—
111	MS	—	+	—	—
115	MS	—	+	—	—
116	MS	—	+	—	—
117	MS	—	+	—	—
119	MS	—	+	—	—
Group 2					
53	CM	+	—	—	—
66	CM	+	ac[a]	—	ac[a]
72	CM	—	+	+	—
87	CM	—	+	—	+
93	CM	—	—	+	—
97	CM	—	—	—	+
Group 3					
15	RA	—	—	+	—
18	RA	—	—	+	—
23	SLE	+	+	+	+
48	spinal meningioma	+	—	—	—

[a] ac = anticomplementary.

TABLE 4

REACTIONS OF ALL SERA POSITIVE WITH SALINE BRAIN EXTRACT

Weak reactions are in parentheses. Samples also positive with lipid antigen are marked with an asterisk.

Tissue homogenates																			
serum No.	diagnosis	Cerebrum	Cerebellum	Pons	Medulla oblongata	Semilunar ganglion	Peripheral nerve	Neurohypophysis	Epiphysis	Other organ homogenates with positive reaction	MS brain	Bovine brain	Grey matter	White matter	Activity grey/white matter	Active antigen fraction after separation on Sepharose 4B		Myelin	Resistance to 80 °C
55	MS	+	+	+	+	(+)	—	—	—		+	+	+	+	1:8	V_0		+	—
76*	MS	+	+	+	+	(+)	—	—	—	(adenohypophysis)	+	+	+	+	1:8	V_0		+	—
82	MS	+	+	+	+	—	—	—	—		+	—	+	+	1:4	V_0		+	—
100	MS	+	+	+	+	(+)	—	—	—		+	+	+	+	1:4	V_0		+	—
101	MS	+	+	+	(+)	(+)	—	+	(+)	(plexus chorioideus), (thymus), (adenohypophysis), (prostate), (placenta), (kidney)	+	+	+	+	4:1	V_0		—	—
A13*	MS	+	+	+	+	+	+	—	—	(gall bladder), (kidney)	+	+	+	+	1:16	V_0		+	+
53	CM	(+)	(+)	(+)	(+)	—	—	—	—										
66	CM	+	+	+	+	(+)	—	—	—	(kidney)	+	+	—	+		V_0			—
23*	SLE	(+)	(+)	—	—	(+)	—	—	—	(thymus), (testis), (prostate), (kidney), (spleen), (lung)									
48	spinal meningioma	+	+	+	+	(+)	—	+	(+)	(plexus chorioideus), (thymus), adenohypophysis, (testis), prostate, placenta, kidney, spleen		+	+	—		V_0			—

TABLE 5

REACTIONS OF SOME CSF POSITIVE WITH SALINE BRAIN EXTRACT

Weak reactions are in parentheses. Samples also positive with lipid antigen are marked with an asterisk.

Tissue homogenates																
CSF No.	diagnosis	Cerebrum	Semilunar ganglion	Peripheral nerve	Neurohypophysis	Myocardium	Spleen	Kidney	MS brain	Bovine brain	Grey matter	White matter	Activity grey/white matter	Active fraction after separation of antigen on Sepharose 4B	Myelin	Resistance to 80 °C
8	MS	+	(+)	—	—	—	—	—	+	+	+	+	1:8		+	—
12	MS	+		—	—	—	—	—	+	+	+	+	1:4		+	—
14*	MS	+	+	—	—	—	—	—	+	+	+	+	>1:1		—	—
27	MS	+	(+)	—	—			—	+	+	+	+			+	
28	MS	+	+	—	—	—	—	—	+	+	+	+	1:1	V_0	—	—
33	MS	+	+	—	—	—	—	—	+	+	+	+		V_0	+	—
38	MS	+	+	—	—	—	—	—	+	+	+	+	1:8	V_0	+	—
46*	MS	+	+	—	(+)	—	—	(+)	+	+	+	+			+	+
47	MS	+		—	—	—	—	—	+	+	+	+	1:4			
55	MS	+	+	—	—			—	+	+	+	+	1:8	V_0	+	—
64	MS	+	+	—	—			—	+	+	+	+	1:4	V_0	+	—
75	MS	+	+	—	—			—	+	—	+	+	1:8	V_0	+	—
76	MS	+	(+)	—	—	—	—	—	+	+	+	+	1:4	V_0	+	—
78	MS	+	+	—	—			—	+	+	+	+	1:4		+	—
90	MS	+	+	—	—			—	+	+	+	+			+	
96	MS	+	+	—	(+)			—	+	+	+	+	1:4	V_0	+	—
100	MS	+	+	—	—			—	+	+	+	+	1:16	V_0	+	—
108	MS	+	(+)	—	—			—	+	+	+	+	1:8		+	
115	MS	+	(+)	—	—			—	+	+	+	+	>1:1		—	
116	MS	+		—	—	—	—	—	+	+	+	+	1:4		+	—
117	MS	+	+	—	—			—	+	+	+	+			+	
119	MS	+	(+)	—	—			—	+	+	+	+			+	
72	CM	+	(+)	—	—			—	+	+	+	+				
87*	CM	+	+	—	—			—	+	+	+	+				

TABLE 6

CALCULATED MINIMUM ANTIGENIC ACTIVITY OF GREY AND WHITE MATTER HOMOGENATE COMPARED WITH HOMOGENATE OF TISSUES WITH NEGATIVE REACTION

	Grey matter	White matter
Serum No.		
55	4 : 1	32 : 1
76	2 : 1	16 : 1
82	1 : 1	4 : 1
100	4 : 1	16 : 1
101	2 : 1	1 : 1
A13	8 : 1	128 : 1
CSF No.		
8	2 : 1	16 : 1
28	8 : 1	8 : 1
38	8 : 1	64 : 1
55	8 : 1	64 : 1
64	4 : 1	16 : 1
75	1 : 1	8 : 1
76	4 : 1	16 : 1
78	1 : 1	4 : 1
96	64 : 1	256 : 1
100	8 : 1	128 : 1
106	8 : 1	64 : 1

The reactions with saline extracts of nervous tissue were, in general, specific to this tissue. However, serum 23 from a SLE patient, serum 48 from a patient with a spinal meningioma and serum 101 from a MS patient gave positive reactions also with a number of other tissues. It is notable that the two latter sera reacted preferentially with grey brain matter. Absorption experiments with serum 101 indicated that the reactive antigen in grey matter was present also in neurohypophysis but not in kidney. Absorptions with serum 76 indicated the non-identity of the reactive antigen in white matter and adenohypophysis. This serum was from a MS patient who had been repeatedly treated with injections of porcine ACTH and who had reacted with an anaphylactoid reaction to an intramuscular injection of porcine ACTH 8 months before the studied serum sample was obtained.

In some instances the minimum degree of nerve tissue specificity of the antigens could be calculated. The results were limited by the dilutions at which the serum and CSF samples had to be tested. Probably most antigen titrations were done below the preferable region of antibody excess. Table 6 gives the results.

Findings with semilunar ganglion and trigeminal nerve

Serum and CSF samples with positive reaction to saline brain extract were generally reactive with homogenates of semilunar ganglion (Tables 4 and 5), but the results were not always reproducible. This could be because of difficulties in obtaining representative tissue samples. Therefore homogenates of carefully dissected semilunar

ganglion were tested together with homogenates of the nerve proximal and distal to the ganglion. Sera 55, 66 and 100 and CSF 46, 55, 64, 96 and 100 gave positive reactions only with the proximal part (i.e. the root) of the trigeminal nerve and not with the ganglion or the distal branches. Therefore it seems probable that the positive reactions often obtained with semilunar ganglion depend on contamination with the proximal part of the nerve and not on antigen present in the ganglion itself. The nonreactivity of the nerve distal to the ganglion was in agreement with the findings with femoral nerve.

Grey–white matter differences (Tables 4 and 5)

Positive serum and CSF samples were tested with serial 2-fold dilutions of 1:50 (w/v) homogenates in saline of frontal cortex and centrum semiovale. Most samples gave positive reactions with both types of homogenate, but the antigenic activity was 4–16 times higher in white matter homogenate (WMH). Serum from one MS patient (101) and from one with a spinal meningioma (48) reacted preferentially with grey matter homogenate (GMH). Titration against the former serum showed the antigenic activity to be about 4 times higher in grey matter than in white matter. Against CSF 28 both types of homogenate showed equal antigenic titre. Absorption of this CSF with WMH extinguished reactivity with both WMH and GMH, indicating the identity of the antigen in these tissues. Frontal cortex and nucleus caudatus, on the one hand, and centrum semiovale and corpus callosum, on the other, were compared for antigenic activity in tests with representative MS sera. These sources of grey and of white matter were equivalent in these experiments.

Allogeneic and species differences

In order to reveal reactions to antigens not generally present in normal human brain or in MS brain and to test the species specificity, the following experiment was made. Sera and CSF with positive reaction to saline brain extract were tested with a panel of saline extracts of 5 human brains without macroscopic pathological involvement, one brain with advanced MS involvement, and one bovine brain. Paired serum and CSF samples from 8 MS patients with negative reactions in the screen test were also tested with these extracts. Of the previously positive samples, most gave positive reactions with the whole panel of extracts, whereas one serum (82) and one CSF (75) gave positive reactions only with the human brain extracts (Tables 4 and 5). Extended experiments with serum 82 could not reveal any reaction with bovine brain homogenate, the activity of this antigen being at least 32 times higher in human brain. In other tests, these samples did not differ from most of the positive samples. The corresponding antigen is species restricted rather than species specific, as serum 82 and CSF 75 gave positive reactions with rat brain. Of the previously negative samples, all gave negative results with the whole panel of extracts. As several brains were used in the various tests, many MS sera and MS CSF were tested with 5–12 individual "normal" human brains. No human brain was found that gave negative reactions with any of the serum and CSF samples with established positive reactions to other human brains.

Testing samples of serum and CSF against fractions of saline brain extracts obtained after gel filtration on Sepharose in 4B showed that the antigenic activities were eluted in the fractions of the first protein peak corresponding to the void volume (Vo) (Tables 4 and 5). As this medium has an exclusion limit at a molecular (particle) weight of several millions, this probably means that the studied antigens are not in true solution in the saline extract.

Myelin

The organ specificity, the distribution within the nervous system and the gel filtration properties indicated that some of the antigens might be associated with myelin of CNS, whereas the specificity of one MS serum (101) and serum from a patient with a spinal meningioma (42) was evidently not. Myelin pelleted in 0.9% NaCl by centrifugation at 2000 $\times$ g_{max} for 30 min was resuspended in the proportions 1:50 (v/v) in 0.9% NaCl and tested as antigen against a number of sera and CSF reactive with saline brain extracts. Tables 4 and 5 give the results with all the sera and most of the CSF tested in this way. Twenty-nine of the 32 MS CSF with positive reaction against saline brain extract were positive against myelin and 3 were negative. Among the negative samples was CSF 28 which reacted with an antigen with equal distribution between grey and white matter and serum 101 with a preferential reactivity with grey matter. Several samples with negative reaction with saline brain extract were upon retesting also negative with myelin as antigen. Calculated on a weight basis lyophilized myelin exhibited the same degree of antigenic activity with a number of representative sera (100 and A 13) and CSF (38, 46, 55, 96, 100 and 108) as the lyophilized water insoluble fraction of WMH, indicating that much, but possibly not all, of the activity in the white matter is caused by myelin. In serum 55, 76, 82, 100 and A 13 and CSF 38, 55, 64 and 100 antibody activity against GMH and WMH could be absorbed out by CNS myelin. This indicates that myelin is associated with all the antigens involved in these reactions, and that the reactions of these samples with grey matter are not caused by separate antigens. The same experiment performed with serum 101 did not significantly reduce the titre against GMH and WMH.

Heating whole brain homogenate (1:50, w/v) to 80 °C for 10 min caused a complete loss of antigenic activity against most sera and CSF. Only the reactivity with serum A 13 with sulfatide antibodies and CSF 46 with galactosylceramide antibodies resisted this treatment, although a slight loss of activity could be discerned (Tables 4 and 5). The antigenic activity to serum 55, 76, 82 and 100 was resistant to 50 °C for 10 min, but was markedly affected by 60 °C for 10 min. The antigen reactive with serum 101 was destroyed by heating to 50 °C for 10 min.

Lipid antigens

Seven sera and 7 CSF from 13 patients were positive against TLA from brain (Tables 2 and 3). These and additional samples from the same patients were further characterized in a number of tests. Lack of material again limited the number of experiments, especially for CSF. Table 7 summarizes the results. As far as tested, the samples positive to brain lipid extracts were not organ specific in reaction when

TABLE 7

REACTION OF ALL SAMPLES POSITIVE WITH TLA FROM BRAIN

Weak reactions are in parentheses. Samples also positive with saline brain extract are marked with an asterisk.

		Antigens								
	Diagnosis	TLA from brain	TLA from peripheral nerve	TLA from liver	TLA from kidney	TLA from spleen	TLA from lung	Hydrolysis resistant brain lipids + auxiliary lipids	Lecithin–cholesterol	Reacting TLC fractions
Serum No.										
64	MS	+	(+)	+	(+)	(+)	—	+	(+)	multiple
76*	MS	+		+	(+)			+	—	—
A13*	MS	+	+	+	+	+	+	+	—	sulfatide
72	CM	+	—	+	+	+	—	+	+	multiple
93	CM	+	+	+	+	+	—	+	+	multiple
15	RA	+	+	+	(+)	+	(+)	+	—	—
18	RA	+	+	+	+	+	+	+	+	multiple
23*	SLE	+	+	+	+	+	—	+	+	multiple
CSF No.										
14*	MS	+						+	(+)	
16	MS	+							—	cerebroside
46*	MS	+	(+)	(+)	(+)	—	—		—	cerebroside; X-type
77	MS	+							—	sulfatide
87*	CM	+							—	(cerebroside); C-type
97	CM	+								
23*	SLE	+								

tested against TLA from a number of organs. It was not tested whether or not the reactions with the different organ extracts were caused by the same antigens (haptens).

Most of the tested samples were positive with brain lipids resistant to mild alkaline hydrolysis when 150 μg of these lipids were combined with 100–200 μg of auxiliary lipids (lecithin–cholesterol, 1:1 or 1:3, w/w). This finding indicated that the involved haptens are not glycerol ester lipids. Tests with these lipids without auxiliary lipids were negative. By testing first with rough fractions and then with successively purer fractions, all with added lecithin and cholesterol, a few samples were found that had positive reactions with cerebroside and sulfatide. With two serum samples positive against TLA from brain and brain lipids resistant to mild alkaline hydrolysis the reaction could not be traced to the TLC fractions, although a wide spectrum of auxiliary lipid combinations was used. Five serum samples representing different diagnoses gave positive reactions with most, but not all, TLC fractions. However, these samples were also positive with the auxiliary lipids, the suggestive patterns of positive and ne-

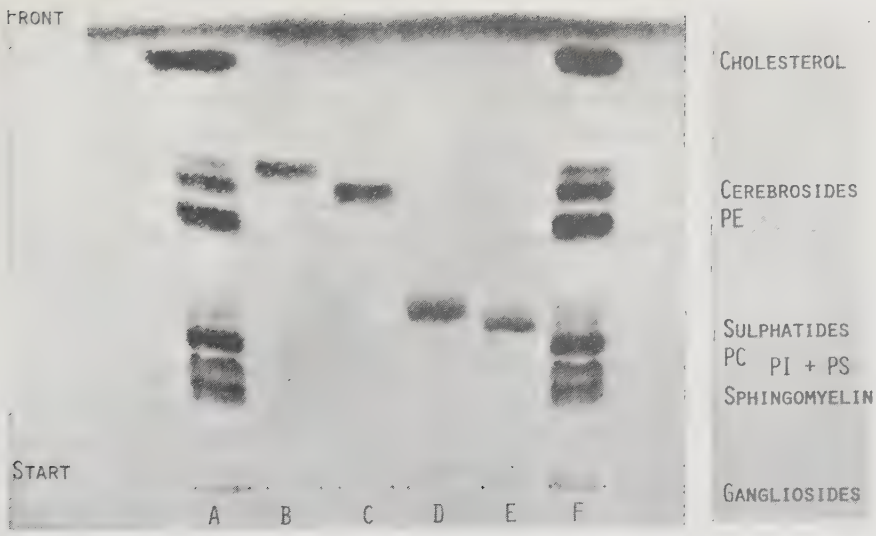

Fig. 2. Thin layer chromatogram of Folch-Pi extract of normal human brain (A and F), cerebroside with normal fatty acid residue (B), cerebroside with hydroxy fatty acid residue (C), sulfatide with normal fatty acid residue (D), sulfatide with hydroxy fatty acid residue (E). PE – phosphatidylethanolamine, PC – phosphatidylcholine, PI – phosphatidylinositol, PS – phosphatidylserine. The absorbent was silica gel H; the solvent was chloroform–methanol–water (65:25:4, v/v/v).

gative reactions with the TLC fractions being the result of the inhibitory activity of certain lipids, for instance, sphingomyelin and lecithin in excess. When tested with cerebroside–lecithin–cholesterol antigens in the proportions 1:2.2:6.75 and 1:50:50 (w/w/w) CSF 46 reacted only with the first antigen and CSF 87 only with the second antigen. They therefore contain antibodies to cerebroside of X-type and of C-type according to the terminology of Rapport, Cavanna and Graf (1967).

In experiments with serum A 13 an antigen with sulfatide–lecithin–cholesterol in the proportions 1:12.5:12.5 (w/w/w) was close to optimum, in the sense that it had a low anticomplementary activity and a high antigenic titre. Sulfatide in this system could be detected at a concentration of 0.3 mg/l corresponding to 9 ng per test. For the complete antigen the figures are 8 mg/l and 230 ng, which is comparable with the figures obtained with lyophilized myelin. CSF 46 showed no reaction with glucosylceramide in a number of auxiliary lipid combinations, but gave positive reactions with both chromatographical subfractions of brain cerebroside (Fig. 2, B and C), i.e. with normal and with hydroxy fatty acid residue. Serum A 13 showed equal reactivity with the corresponding subfractions of sulfatide (Fig. 2, D and E). In order to assess the specificity of the reactions with the cerebroside antigen, a sulfatide sample was subjected to mild acid hydrolysis. During the reaction the sample lost its reactivity with the serum (A 13) with known positive reaction to this antigen and, instead, obtained reactivity with a CSF (46) with antibodies to cerebroside. This change in immunological reactivity was concomitant to the disappearance of the sulfatide spot and the appear-

ance of the cerebroside spots on TLC. The identity of the newly-formed antigen was checked by testing TLC fractions of the reaction products.

The reaction between some sera and lecithin–cholesterol without further lipids was studied by the aid of serum 93. Some of the experiments included serum 23 and 72 which gave essentially the same results as serum 93. To see whether the reaction was caused by a contamination or other factor in a certain batch of lecithin or cholesterol, a series of combinations of auxiliary lipids from different sources were tested. Lecithin prepared from human brain by TLC and the lecithin and cholesterol preparations listed under "Chemicals" were used. In addition sphingomyelin from sheep erythrocytes (Supelco) was used as an alternative to lecithin (Niediek and Pette 1963; Kinsky 1972). Some differences in reactivity were registered between the various batches of lecithin (egg lecithin $>$ brain lecithin $\approx$ synthetic lecithins $>$ soybean lecithin), but generally positive reactions were observed with auxiliary lipid compositions of 1 part lecithin or sphingomyelin to 2 or more parts cholesterol (w/w) at a concentration of 25–100 μg/ml. Sphingomyelin formed an antigen that was about twice as potent as the corresponding (w/w or mol/mol) lecithin-based antigen. In preliminary experiments cholesterol mixed with ceramide, galactocerebrosides, glucocerebrosides, and phosphatidyl ethanolamine, respectively, also gave positive reactions. Tested separately the examined lipids gave negative reactions.

Two out of 3 patients with combined RA and compressive myelopathy were positive with TLA and brain lipids resistant to mild alkaline hydrolysis. The sera reacted also with similar extracts from other organs and one of them (18) reacted with lecithin–cholesterol mixtures. Similar results were obtained in 5 RA patients with compressive myelopathy further tested, but not in 5 RA patients without neurological disease (Results to be published elsewhere). Serum from the SLE patient (23) gave positive reactions with a broad range of lipid compositions, including lecithin–cholesterol (1:3, w/w).

A few samples were positive both with the saline extract and lipid antigens from brain (Tables 3, 4, 5 and 7). The relative strength of the antigen preparations and the temperature stability of the whole brain homogenate suggested that the reactions with serum A 13 and CSF 46 were caused by the lipid haptens and that the reactions with serum 76 and CSF 87 were caused by lipid haptens and some nonlipid antigen. Patients 64 and 72 were exceptional in having autoantibodies of different specificities in serum and CSF. In both cases serum reacted with lecithin–cholesterol mixtures.

Ig class and titre of the antibodies

The Ig class of antibodies in positive sera could be determined by testing of fractions after gel filtration on Sephadex G-200 as only IgM and IgG are complement-fixing (Fig. 1). In MS all serum reactions with saline brain extracts were found to be mediated by IgG. Three sera reactive with lipid antigen were examined in this way. The lipid antibodies in serum 76 were of IgG class. The activity in serum 93 was eluted in the IgG-rich fractions and to a slight extent in the IgM-rich fractions. No activity was eluted before IgM on gel filtration of this serum on Sepharose 4 B. The sulfatide antibodies in serum A 13 were of IgG class, but had been of both IgG and IgM class

in an earlier obtained sample. This might possibly indicate a more recent antigenic stimulation in the earlier sample.

Paired serum and CSF samples from patients 55, 76, and 100 were serially diluted to 1000, 500, 250, 125, 60, 30, 15, and 7 mg IgG/l and tested with WMH (1:50, w/v). The lowest dilution to give a positive reaction was for the sera 125, 125, and 500 mg/l, respectively, and for the CSF samples 30, 30, and 250 mg IgG/l, respectively. Thus the antibody activity related to IgG content was 2–4 times higher in CSF than in serum in these patients. In several MS patients with serum that was negative at 1000 mg IgG/l, CSF gave strong reactions at 500 mg IgG/l and although end point titrations were not performed, it was observed that, in patients 14, 33, 46, 64, 96, and 108, CSF was positive at IgG concentrations of 250–50 mg/l. All but two (77 and 82) MS patients with positive reactions had two or more M-components in the gamma region after agar gel electrophoresis. In CM positive reactions in CSF were found only in patients with this abnormality, whereas positive serum reactions were found also in patients without M-components in CSF (53 and 93).

Survey

From the findings with individual MS and CM samples the following survey of involved autoantigens was compiled. Several of the indicated specificities might prove to be heterogeneous. Representative sera and CSF are also presented.

I. Associated with CNS myelin. Organ specific. Labile to heat. Not solubilized by saline or distilled water. Nonlipid. Not species specific. Reacts with serum 55, 66 (?), 76, 100 and CSF 8, 12, 27, 33, 38, 47, 55, 64, 76, 78, 90, 96, 100, 108, 116, 117, 119.

II. As I, but species restricted. Might represent a different determinant on the same molecule. Serum 82 and CSF 75.

III. Equally distributed between grey and white matter. Organ specific. Labile to heat. Not solubilized by saline. Nonlipid. CSF 28.

IV. Predominantly in grey matter. Probably present in neurohypophysis and epiphysis and possibly in some nonneural tissues. Labile to heat. Not solubilized by saline. Nonlipid. Serum 48 (?) and 101.

V. Cerebroside (galactosylceramide). According to the literature mainly represented in myelin (O'Brien and Sampson 1965). Immunologically detectable in saline suspensions of brain tissue (and kidney) and in lipids extracted from neural and possibly some nonneural tissues. Heat resistant. CSF 16, 46 (X-type) and 87 (C-type).

VI. Sulfatide (galactosylceramide-3-sulfate). According to the literature concentrated in myelin (O'Brien and Sampson 1965), but found elsewhere in the body. Immunologically detectable in suspensions of central and peripheral nervous tissue, gall bladder, and kidney, and in lipid extracts from many tissues. Stabile to heat. Serum A 13 and CSF 77.

VII. Cholesterol in combination with a variety of lipids with different degrees of polarity. Serum 18, 23, 72, 93 and CSF 14 (?).

It may be calculated that antigen I gives positive reactions with about 5% of the MS sera and about 40% (47% if the anticomplementary samples are excluded) of the MS CSF. The other antigens together give about 3% positive reactions with MS sera and about 12% (14%) positive reactions with MS CSF.

DISCUSSION

The screening

The principle of screening all serum and CSF samples at the same IgG level, 1000 mg/l, was retained in this investigation from an earlier study (Ryberg 1976). As it was shown that antibodies in MS CSF against antigen in saline brain extracts

are of IgG class, this method of testing gives evidence of an intrathecal synthesis of these antibodies in cases with a positive reaction in CSF and a negative one in serum. In cases with positive reactions in both serum and CSF, the IgG antibody might have been transferred from serum to CSF (Frick and Scheid-Seydel 1958; Fossan 1977). However, the 3 MS patients in this study with positive reactions to saline brain extract in both serum and CSF had a 2–4 times higher antibody/IgG ratio in CSF, indicating that at least part of the CSF antibody was locally produced. The situation of a positive reaction in serum and a negative in CSF might theoretically be due to an intrathecal synthesis of large amounts of IgG of different specificities or the IgM nature of the serum antibody. The latter explanation was, however, not applicable to any of the MS cases. Also, a hypothetical specific increase in the elimination rate (e.g. by an absorption in vivo) in serum or CSF might influence the antibody/IgG ratio in the two compartments.

The present study has confirmed the finding of complement-fixing antibodies to antigens in saline brain extracts in frequent CSF samples and in occasional serum samples from MS patients (Ryberg 1976). It has, in part, been extended to patients with CM, a heterogenous patient group that includes numerous patients that at autopsy prove to have MS (Marshall 1955), as can often be suspected from CSF findings (Gårde and Kjellin 1971; Link, Norrby and Olsson 1976) and neurophysiological phenomena (Halliday, McDonald and Mushin 1973; Bynke, Olsson and Rosén 1977). In contrast, antibodies to saline brain extract were detected in only 2 out of 60 control cases; in serum and CSF from a patient with SLE, a condition known for its association with autoantibodies of many kinds (Hughes and Lachmann 1975), and in serum from a patient with a meningioma compressing the spinal cord. In these samples, the reaction pattern was distinct from that of most MS samples.

Antigens in saline extracts

Among the antibodies to saline brain extracts 6 separate specificities could be discerned by a few fundamental experiments with MS samples. Four of them were directed to myelin antigens, two of which were glycolipids. Most positive reactions were caused by some nonlipid component associated with CNS myelin, which is labile to heat and is not solubilized by saline or distilled water. Possibly the antigen denoted II (see p. 373) is a species-restricted determinant belonging to this component. Further research is needed to learn whether the component is homogeneous or can be dissected into separate antigens.

The extinction of some of the antigenic activities in whole brain homogenate by heating to 50–80 °C suggests that the corresponding determinants are of protein nature, as this treatment usually leaves carbohydrate antigenic determinants intact (Davies 1973). However, as the antigens were not purified but probably integrated in membrane structures, the availability of the determinants might also have been reduced by change in the membrane architecture or denaturation of neighbouring molecules.

Aarli, Aparicio, Lumsden and Tönder (1975) demonstrated an unspecific affinity between the Fc part of normal human IgG and myelin. The restricted occurrence of

the antibodies studied here and the diversity of their specificities indicate a different mechanism for their interaction, probably a true antigen–antibody reaction. The same authors also claim that the IgG binding component in myelin is the basic protein. The evidence for this claim is rather indirect, and several investigations have failed to detect antibodies to basic protein with radioimmune methods in samples from MS patients and healthy controls (Lisak, Heinze, Falk and Kies 1968; Lennon and Mackay 1972; Schmid, Thomas, Hempel and Grüninger 1974). Therefore it is improbable that antibodies to myelin basic protein are responsible for any appreciable part of the myelin reactions in this material.

Antigens I to VI were detected in all of a number of tested human brain homogenates, including homogenate of one MS brain. They therefore seem to be normally present in human brain and are not necessarily absent from or blocked in MS brain as a whole. Thus it is possible here to speak of autoimmune phenomena with some justification.

The finding of antigen typical for CNS myelin in the trigeminal nerve proximal to ganglion semilunare is of interest as the proximal segments of the nerve roots are the only parts of the peripheral nervous system to be affected by MS plaques (Lumsden 1972).

Lipid antigens

The experiments with lipid antigens also revealed a number of antibody specificities in MS and CM patients. Two of the antigens (haptens) could be chemically characterized as cerebroside and sulfatide. An unexpected reactivity to lecithin–cholesterol and sphingomyelin–cholesterol mixtures was also detected in a few samples from patients with MS, CM, RA, and SLE. Lipids are known to depend on "auxiliary lipids" (Pangborn 1941; Rapport and Graf 1969) or other carrier molecules (Taketomi and Yamakawa 1963; Teitelbaum, Arnon, Sela, Rabinsohn and Shapiro 1973) for their function in immunological tests. Although several lipids can serve as auxiliary lipids, a lecithin–cholesterol mixture has been used by most authors in the complement-fixation technique. Lecithin has the double effect of increasing antigenic activity and eliminating anticomplementarity, which is a prominent feature of some purified lipids (Rapport 1967). Cholesterol seems to improve the complement-fixing antigenic activity by conferring rigidity to the complexes formed by hapten and auxiliary lipids (Humphries and McConnell 1975). Advantage of this information was taken in composing a potent "total lipid antigen" from brain to screen for samples with antibodies to lipids and in composing suitable test antigens from lipid fractions. The problem of finding the optimum hapten–auxiliary lipid composition in every possible hapten–antibody system, however, is great, especially with an antibody source as scanty as CSF. Indeed, antibodies to a defined hapten can have different requirements for the hapten–auxiliary lipid formulation. This has been shown in rabbit antibodies to galactosylceramide (Rapport et al. 1967), and has also been found in this study. It is thus possible that a more extensive test with different hapten–auxiliary lipid proportions might reveal more samples positive to known haptens and new antibody specificities. The dominating cerebroside of adult human brain is galactosylceramide (Suzuki and Suzuki 1972).

Immunologically it has been found to be a major nerve tissue specific, species non-specific hapten (Niedieck and Lanken 1961; Joffe, Rapport and Graf 1963), but has chemically been demonstrated in low amount in kidney (Makita 1964). The naturally occurring substance is only homogeneous with respect to the polar moiety. The ceramide part demonstrates some variability (Suzuki and Suzuki 1972).

Tests with cerebrosides substituted in different ways have shown the galactose moiety to be the immunodominant part of the molecule (Joffe et al. 1963; Niedieck 1975). Inhibition experiments with various simple and derived sugars (Rapport and Graf 1969; Duponey 1972; Niedieck 1975) and the cross reactivity between cerebrosides and galactodiglyceride (Duponey 1972; Niedieck 1975) and certain gangliosides (Alving, Fowble and Joseph 1974) further indicate the central role of the β-glycosidically-bound galactose. Probably the hydrophilic part carries the immunological specificity in all glycolipid haptens, but the chain length of the fatty acid residue of a glycosphingolipid can have a profound quantitative influence on its antigenic activity (Graf and Rapport 1974).

Galactosylceramide is not encephalitogenic, but rabbit antiserum to this substance is claimed to demyelinate CNS cultures (Dubois-Dalcq, Niedieck and Buyse 1970). Fry, Weissbarth, Lehrer and Bornstein (1974) found that the in vitro myelination inhibiting and demyelinating capacity of rabbit antisera to white matter could be eliminated by absorption with a mixture of cerebroside and lecithin. The same investigators also showed a reduced sulfatide synthesis in developing CNS cultures exposed to antiserum to cerebroside. Similar results were also seen with rabbit antisera to synthetic galactosylceramide (Hruby, Alvord and Seil 1977). So far, attempts to reproduce these phenomena in rats (Seil, Smith, Leiman and Kelly 1975) and guinea pigs (Lebar, Boutry, Vincent, Robineaux and Voisin 1976) have failed.

In this study, the antibodies to cerebroside showed no reactivity with glucosylceramide. As this molecular species differs from galactosylceramide in the epimeric configuration at the C-4 position of the hexose, we have a strong indication that this is an essential part of the antigenic determinant also in this antigen-antibodysystem. No difference was seen in the antigenic activity of the two principal chromatographical subfractions of cerebroside; kerasin, with a normal fatty acid residue, and phrenosin, with an —OH group on the C-2 position of the fatty acid residue. This might be explained by antibodies with two different specificities or by an antibody without the ability to detect this difference. The latter possibility is supported by the finding of antibodies with cross reactivity to these subtypes of cerebroside in animals immunized with spinal cord (Niedieck 1975).

Antibodies to cerebroside have been demonstrated with different techniques in sera from a variety of persons, including MS patients and normal subjects (Duponey 1972; Lisak, Zwiman and Norman 1975; Hirsch and Parks 1976). They have been found in the CSF of rabbits with experimental allergic encephalomyelitis induced by emulsions of homologous spinal cord in complete Freund's adjuvant (Kuwert and Niedieck 1965), but do not appear to have been earlier reported in human CSF.

The sulfatide present in nervous tissue is the sulfate ester of galactosylceramide. The negatively charged sulfate group is bound to the C-3 position of the galactose

moiety causing a marked change in the charge and outline of the immunologically critical part of the molecule. Sulfatide exhibits a molecular heterogeneity in its ceramide part similar to that of galactosylceramide (Suzuki and Suzuki 1972). At low salt concentrations, the negatively charged sulfatide gives nonspecific precipitates with serum proteins (Niedieck 1967) and has strong anticomplementary properties (Niedieck 1967; Rapport and Graf 1967). From the negative controls for anticomplementarity and from the negative results in most of the samples, it is seen that the positive reactions are not due to these properties of the antigen. In analogy with galactosylceramide, no difference in the antigenic activity of the two chromatographical subfractions of sulfatide was seen. The immunogenic capacity of sulfatide has recently been demonstrated (Hakomori 1974), but there seems to be no earlier report of antibodies to this hapten in man. It is interesting that sulfatide appears to fulfil several of the requirements of an opiate receptor (Loh, Cho, Wu, Harris and Way 1975) and seems to be in some way coupled to Na^+–K^+-ATPase (Karlsson et al. 1971, 1973). An antibody interacting with sulfatide involved in these putative functions might have physiological effects and thus contribute to the symptomatology in certain patients. However, the two patients with this antibody did not exhibit any unexpected clinical features.

Why are antibodies formed to the normal body constituents galactosylceramide and sulfatide? As haptens, these substances are dependent on a carrier for their immune response (Leskowitz 1972). Both endogenous carriers, such as "hidden antigens" or denaturated substances and exogenous carriers (nutritional, infectious, or iatrogenically introduced), should be considered. For example, the lipid-containing RNA viruses are potential inducers of autoimmune phenomena by this and other mechanisms (Drzeniek and Rott 1969). As seen from the results in the control material, the mere destruction of nervous and other tissue is not a sufficient prerequisite for a positive reaction in this assay. However, the existence of antibodies with affinity to these glycolipids does not necessarily imply the immunogenic role of these substances. Structures similar to or identical with the relatively simple antigenic determinants are probably present in a number of endo- and exogenous substances. Thus galactodiglyceride (Duponey 1972), and a not specified ganglioside (Alving, Fowble and Joseph 1974), can crossreact with cerebroside in vitro.

The finding of positive reactions with mixtures of cholesterol and other lipids, among them lecithin, reveals a potential pitfall in serological tests which employ a lecithin–cholesterol mixture as auxiliary lipids, for example the Wasserman reaction. The phenomenon is open to several interpretations:

(1) Antibodies to cholesterol. Spontaneous occurrence is not documented, but such antibodies have been experimentally induced (Sato and Hara 1972).

(2) Antibodies to some antigen (hapten) contaminating the tested lipids. The varying origin of these lipids does not favour such an interpretation.

(3) Alving, Richards and Guirguis (1977) demonstrated a factor in human serum that, in the presence of cholesterol-rich liposomes, activates complement by the classical pathway. However, this factor was not reactive with sphingomyelin–cholesterol liposomes.

(4) Weissman, Brand and Franklin (1974) found an interaction between heat aggregated Ig and lecithin-cholesterol liposomes. The active factor in the present experiments, however, was mainly

of a size corresponding to monomeric IgG, and the phenomenon was limited to a few serum and CSF samples.

(5) C-reactive protein is able to react with lecithin and sphingomyelin under consumption of complement (Kaplan and Volanakis 1974). However, the activation by this mechanism of guinea pig complement, which was used in the complement fixation test, needs the presence of human C 1q (Volanakis and Kaplan 1974); this factor was routinely inactivated by heating the samples.

(6) A non specific affinity between certain antibodies and aggregates of certain lipids. Possibly such an affinity might be mediated by the previous formation of soluble immune complexes in vivo. Several amphipathic lipids are known to spontaneously bind to biological membranes (Marcus and Cass 1969) or the artificial membranes of liposomes (Kinsky 1975) without losing immunological availability of the hydrophilic determinant. Therefore amphipathic haptens bound to immunoglobulins by their hydrophilic immunodominant part and with the hydrophobic part protruding might hypothetically bind in a non specific way to micelles, liposomes and natural membranes by a similar mechanism. Monomeric IgG-hapten complexes of the kind discussed here are not complement activating (Jaton, Huser, Riesen, Schlessinger and Givol 1976) and will not be detected in a test for anticomplementary activity. But if a sufficient number of IgG molecules are brought together in the manner suggested, complement fixation can be expected to take place as though antibodies were bound to liposomes previously sensitized with an appropriate hapten.

Yokoyama, Trams and Brady (1962) reported the finding of antibodies to gangliosides in serum in 20% of a MS material. This observation was recently supported by Hirsch and Parks (1976). The TLA from brain and the brain lipid fraction resistant to mild alkaline hydrolysis employed here contained gangliosides detectable by a specific spray reagent after TLC, but they were lost during the extraction procedure used in preparatory TLC. This might be one reason for some samples that were positive with the cruder lipid antigens being negative with TLC fractions.

With properly composed lipid antigens, the complement-fixation test gives a very sensitive system for the detection of lipid haptens. This probably accounts for the limited organ specificity of the reactions between TLA from different sources and samples with known antibody to galactosylceramide and sulfatide. The simultaneous occurrence, in these samples, of antibodies to lipids not present in brain cannot however, be ruled out with the data available. Only part of the samples with demonstrated antilipid antibodies gave positive reactions with the saline brain extract. Although a saline extract of the crude type used here probably contains most of the antigens in the tissue, even those found in the lipid extract, some of them are evidently difficult to detect immunologically. Perhaps they are present in an insufficient concentration, possibly owing to enzymatic degradation, or perhaps they are hidden in membranes or other subcellular structures. Some haptens are possibly present in a molecular environment that is unfavourable for their immunological reactivity. For example, less than 15% of the galactosylceramide determinants in untreated bovine myelin appear to be serologically reactive (McIlwain, Graf and Rapport 1971).

Why could antibodies to each antigen (hapten) be demonstrated in only a fraction of the MS cases? Possibly, they are synthesized in sufficient amount only in some MS cases or only during certain phases of the disease. On the other hand, it is highly probable that the antibody level is influenced by an absorption of antibody to antigen (hapten) present in serum and CSF or exposed in the tissues (normally or due to barrier damage). Actually, the two identified glycolipid haptens are detectable in normal CSF (Hagberg and Svennerholm 1960; Samuelsson 1971) and in normal serum (Hagberg and Svenner-

holm 1960; Rathke and Jones 1974). MS CSF has increased levels of total cerebrosides (Tourtellotte and Haerer 1969) and sulfatide (Nagai, Kanfer and Tourtellotte 1973). Baumann, Lemonnier, Marteau, Harpin and Lhermitte (1975) found elevated galactosylceramide values in plasma from patients with MS and cerebrovascular diseases. It is not known to what extent the circulating haptens are in an immunologically reactive form. They and possibly other autoantigens might, by competitive binding, influence the amount of antibody available to react with tissue-bound antigen and part of them might well represent antibody-bound material in certain diseases. If so, these complexes might account for some of the anticomplementary activity seen here in 15% of the MS CSF. The IgG accumulated in MS brain (Tourtellotte and Parker 1966), has been suggested to be partly in the form of immune complexes (Woyciechowska and Brzosko 1977). The absence of anticomplementary activity in extracts of MS brain in the present study, indicates that such hypothetical complexes were not of a complement-fixing type, or were present only in very low amount.

It is not known, at the present stage, whether the positive reaction with lipid antigens in certain RA patients is caused by a special character of the RA in these patients, or whether it is caused by an abnormal immunological response to destruction products of the CNS.

CONCLUDING REMARKS

Although the autoantibodies in MS are principally organ specific, they exhibit a marked heterogeneity without any apparent coupling between the various specificities. Such a coupling would have been expected if MS patients had a generally increased tendency to produce autoantibodies. The apparent selectivity might therefore be determined by some property of the individual patient, or it might be a consequence of an immunological heterogeneity in the disease itself or its causative agent(s). A study of the relation between antibrain antibodies and various clinical parameters and CSF findings in MS patients, is under preparation.

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REFERENCES

Aarli, J. A., S. R. Aparicio, C. E. Lumsden and O. Tönder (1975) Binding of normal human IgG to myelin sheaths, glia and neurons, *Immunology*, 28: 171–185.

Åkesson, B., J. Elovson and G. Arvidson (1970) Initial incorporation into rat liver glycerolipids of intraportally injected [^{3}H]glycerol, *Biochim. Biophys. Acta*, 210: 15–27.

Alving, C. R., J. W. Fowble and K. C. Joseph (1974) Comparative properties of four galactosyl lipids as antigens in liposomes, *Immunochemistry*, 11: 475–481.

Alving, C. R., R. L. Richards and A. A. Guirguis (1977) Cholesterol-dependent human complement activation resulting in damage to liposomal membranes, *J. Immunol.*, 118: 342–347.

Baumann, N., M. Lemonnier, C. Jacque, R. Marteau, M. L. Harpin and F. Lhermitte (1975) Plasma galactocerebrosides in multiple sclerosis, *Biomedicine*, 23: 387–390.

Bynke, H., J. E. Olsson and I. Rosén (1977) Diagnostic value of visual evoked response, clinical eye examination and CSF analysis in chronic myelopathy, *Acta neurol. scand.*, 56: 55–69.

Davies, D. A. L. (1973) Preparation of antigens from tissues and fluids. In: D. M. Weir (Ed.), *Handbook of Experimental Immunology*, Blackwell Scientific Publications, Oxford, Chapter 4.

Drzeniek, R. and R. Rott (1969) The possible role of lipid containing RNA viruses for the etiology of autoimmune diseases. In: O. Westphal, H. E. Boch and E. Grundtmann (Eds.), *Current Problems of Immunology (Bayer Symposium I)*, Springer-Verlag, New York, NY, pp. 245–252.

Dupouey, P. (1972) Role of the cerebrosides and a galactodiglyceride in the antigenic cross-reaction between nerve tissue and treponema, *J. Immunol.*, 109: 146–153.

Dubois-Dalcq, M., B. Niedieck and M. Buyse (1970) Action of anti-cerebroside sera on myelinated nervous tissue cultures, *Path. Europ.*, 5: 331–347.

Folch-Pi, J. (1972) Proteolipids. In: A. N. Davison, P. Mandel and J. G. Morgan (Eds.), *Functional and Structural Proteins of the Nervous System*, Plenum Press, New York, NY, pp. 171–199.

Fossan, G. O. (1977) The transfer of IgG from serum to CSF, evaluated by means of a naturally occurring antibody, *Europ. Neurol.*, 15: 231–236.

Frick, E. and L. Scheid-Seydel (1958) Untersuchungen iit J^{131}-markiertem γ-Globulin zur Frage der Abstammung der Liquoreiweisskörper, *Klin. Wschr.*, 36: 857–863.

Fry, J. M., S. Weissbarth, G. M. Lehrer and M. B. Bornstein (1974) Cerebroside antibody inhibits sulfatide synthesis and myelination and demyelinates in cord tissue cultures, *Science*, 183: 540–554.

Fulton, F. and K. R. Dumbell (1949) The serological comparison of influenza virus, *J. gen. Microbiol.*, 3: 97–111.

Gårde, A. and K. G. Kjellin (1971) Diagnostic significance of cerebrospinal fluid examinations in myelopathy, *Acta neurol. scand.*, 47: 555–568.

Gilland, O. (1966) CSF dynamic diagnosis of spinal block, *Acta neurol. scand.*, 42 (Suppl. 21): 45–73.

Graf, L. and M. M. Rapport (1974) Serological activity of glycosphingolipids — Effect of chain length of the fatty acid residue in cytolipin H, *Chem. Phys. Lipids*, 13: 367–371.

Hagberg, B. and L. Svennerholm (1960) Metachromatic leucodystrophy — a generalized lipidosis. Determination of sulfatides in urine, blood plasma and cerebrospinal fluid, *Acta paediat. scand.*, 49: 690–694.

Hakomori, S. (1974) Preparation and properties of anti-sulfatide serum, *J. Immunol.*, 112: 424–426.

Hakomori, S. and B. Siddiqui (1974) Isolation and characterization of glycosphingolipid from animal cells and their membranes. In: S. Fleischer and L. Packer (Eds.), *Methods in Enzymology, Vol. XXXII, (Biomembranes, Part B)*, Academic Press, New York, NY, pp. 345–367.

Halliday, A. M., W. J. McDonald and J. Mushin (1973) Visual evoked response in diagnosis of multiple sclerosis, *Brit. med. J.*, 4: 661–664.

Hallpike, J. F. (1972) Enzyme and protein changes in myelin breakdown and multiple sclerosis, *Progr. Histochem. Cytochem.*, 3: 179–218.

Hirsch, H. E. and M. E. Parks (1976) Serological reactions against glycolipid-sensitised liposomes in multiple sclerosis, *Nature (Lond.)*, 264: 785–787.

Hruby, S., E. C. Alvord, Jr. and F. J. Seil (1977) Synthetic galactocerebrosides evoke myelination-inhibiting antibodies, *Science*, 195: 173–175.

Hughes, G. R. V. and P. J. Lachmann (1975) Systemic lupus erythematosus. In: P. H. Gell, R. R. A. Coombs and P. J. Lachmann (Eds.), *Clinical Aspects of Immunology*, 3rd edition, Blackwell Scientific Publications, Oxford, pp. 1117–1145.

Humphries, G. M. K. and H. M. McConnell (1975) Antigen mobility in membranes and complement-mediated immune attack, *Proc. nat. Acad. Sci. (Wash.)*, 72: 2483–2487.

Jaton, J.-C., H. Huser, W. F. Riesen, J. Schlessinger and D. Givol (1976) The binding of complement by complexes formed between a rabbit antibody and oligosaccharides of increasing size, *J. Immunol.*, 116: 1363–1366.

Joffe, S., M. M. Rapport and L. Graf (1963) Identification of an organ specific lipid hapten in brain, *Nature (Lond.)*, 197: 60–62.

Kaplan, M. H. and J. E. Volanakis (1974) Interaction of C-reactive protein complexes with the complement system. Part 1 (Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin), *J. Immunol.*, 112: 2135–2147.

Karlsson, K.-A., B. E. Samuelsson and G. O. Steen (1971) Lipid pattern and Na^+–K^+-dependent adenosine triphosphatase activity in the salt gland of duck before and after adaptation to hypertonic saline. *J. Membrane Biol.*, 5: 169–184.

Karlsson, K.-A., B. E. Samuelsson and G. O. Steen (1973) The sphingolipid composition of bovine kidney cortex, medulla and papilla, *Biochim. Biophys. Acta*, 316: 317–335.

Karnovsky, M. J. (1965) A formaldehyde–glutaraldehyde fixative of high osmolarity for use in electron microscopy, *J. Cell Biol.*, 27 :137A–138A.

Kates, M. (1972) Separation of lipid mixtures. In: T. S. Work and E. Work (Eds.), *Techniques of Lipidology — Isolation, Analysis and Identification of Lipids*, North-Holland Publishing Company, Amsterdam, pp. 428–446.

Kinsky, S. C. (1972) Antibody-complement interaction with lipid model membranes. *Biochim. Biophys. Acta*, 265: 1–23.

Kinsky, S. C. (1975) Immune reactions of model membranes. In: G. Weissman and R. Claiborne (Eds.), *Cell Membranes. Biochemistry, Cell Biology and Pathology*, H. P. Publishing Co., Inc., New York, NY, pp. 231–238.

Kuwert, E. and B. Niedieck (1965) Anti-cerebroside antibodies in cerebrospinal fluid of rabbits with experimental "allergic" encephalomyelitis, *Nature (Lond.)*, 207: 991–992.

Laatsch, R. H., M. W. Kies, S. Gordon and E. C. Alvord (1962) The encephalomyelitic activity of myelin isolated by ultracentrifugation, *J. exp. Med.*, 115: 777–788.

Laurell, A.-B. and H. Link (1972) Complement-fixing antibrain antibodies in multiple sclerosis, *Acta neurol. scand.*, 48: 461–466.

Lebar, R., J. M. Boutry, C. H. Vincent, R. Robineaux and G. A. Voisin (1976) Studies on autoimmune encephalomyelitis in the guinea pig, *J. Immunol.*, 116: 1439–1446.

Lennon, V. and J. R. Mackay (1972) Binding of ^{125}I myelin basic protein by serum and cerebrospinal fluid, *Clin. exp. Immunol.*, 11: 595–603.

Leskowitz, S. (1972) The immune response to haptens. In: F. Borek (Ed.), *Immunogenicity*, North-Holland Publishing Company, Amsterdam, pp. 131–151.

Link, H. (1973) Comparison of electrophoresis on agar gel and agarose gel in the evaluation of gammaglobulin abnormalities in cerebrospinal fluid and serum in multiple sclerosis, *Clin. chim. Acta*, 46: 383–389.

Link, H., E. Norrby and J. E. Olsson (1976) Immunoglobulin abnormalities and measles response in chronic myelopathy, *Arch. Neurol. (Chic.)*, 33: 26–32.

Link, H., O. Zetterwall and G. Blennow (1972) Individual cerebrospinal fluid (CSF) proteins in the evaluation of increased CSF total protein, *Z. Neurol.*, 203: 119–132.

Lisak, R. P., R. G. Heinze, G. A. Falk and M. W. Kies (1968) Search for anti-encephalitogen antibody in human demyelinative diseases, *Neurology (Minneap.)*, 18: 122–128.

Lisak, R. P., B. Zwiman and M. Norman (1975) Antimyelin antibodies in neurologic diseases — Immunofluorescent demonstration. *Arch. Neurol. (Chic.)*, 32: 163–167.

Loh, H. H., T. M. Cho, Y. C. Wu, R. A. Harris and E. L. Way (1975) Opiate binding to cerebroside sulfate — A model system for opiate-receptor interaction, *Life Sci.*, 16: 1811–1818.

Lumsden, C. E. (1972) An outline of the pathology of multiple sclerosis. In: D. McAlpine, D. F. Lumsden and E. D. Acheson (Eds.), *Multiple Sclerosis — A Reappraisal*, 2nd edition, Churchill Livingstone, Edinburgh and London, pp. 314–321.

Makita, A. (1964) Biochemistry of organ glycolipids, Part 2 (Isolation of human kidney glycolipids) *J. Biochem.*, 55 :269–276.

Mancini, G., J. B. Vaerman, A. O. Carbonara and J. F. Heremans (1964) A single — radial — diffusion method for the immunological quantification of proteins. In: H. Peeters (Ed.), *11th Colloquium on Protides of the Biological Fluids*, Elsevier Publishing Company, Amsterdam, pp. 370–373.

Marcus, D. M. and L. E. Cass (1969) Glycosphingolipids with blood group activity: Uptake by human erythrocytes, *Science*, 164: 553–555.

Marshall, J. (1955) Spastic paraplegia of middle age — A clinico-pathological study, *Lancet*, 1: 643–646.

McIlwain, D. L., L. Graf and M. M. Rapport (1971) Membrane fragments from myelin treated with different detergents, *J. Neurochem.*, 18: 2255–2263.

Müller, R. (1949) Studies on multiple sclerosis, *Acta med. scand.*, 133 (Suppl. 222): 1–214.

Nagai, Y., J. N. Kanfer and W. W. Tourtellotte (1973) Preliminary observations of gangliosides of normal and multiple sclerosis cerebrospinal fluid, *Neurology (Minneap.)*, 23: 945–948.

Niedieck, B. (1967) Immunochemical studies on spinal cord sulfatides, *Z. Immun. Allergie Forsch.*, 132: 139–146.

Niedieck, B. (1975) On a glycolipid hapten of myelin, *Progr. Allergy*, 18: 353–422.
Niedieck, B. and K. Lanken (1961) Zur Serologie der "experimentellen allergischen Neuritis" — Ein Agarpräcipitationstest mit alkoholischen Nervenextrakten, *Klin. Wschr.*, 39:1164–1169.
Niedieck, B. and E. Pette (1963) Immunochemische Untersuchungen zur Identifizierung eines äthanollöslichen Myelinhaptens, *Klin. Wschr.*, 41: 773–778.
O'Brien, J. S. and E. L. Simpson (1965) Lipid composition of the normal human brain: grey matter, white matter, and myelin, *J. Lipid Res.*, 6: 537–544.
Pangborn, M. C. (1941) A new serologically active phospholipid from beef heart, *Proc. Soc. exp. Biol. Med. (N.Y.)*, 48: 484–486.
Rapport, M. M., R. Cavanna and L. Graf (1967) Immunochemical studies of organ and tumour lipids, Part 17 (The existence of two complement-fixing systems involving cerebroside), *J. Neurochem.*, 14: 9–18.
Rapport, M. M. and L. Graf (1967) Preparation and testing of lipids for immunological study. In: C. A. Williams and M. W. Chase (Eds.), *Methods in Immunology and Immunochemistry, Vol. 1*, Academic Press, New York, NY, pp. 187–196.
Rapport, M. M. and L. Graf (1969) Immunochemical reactions of lipids, *Progr. Allergy*, 13: 273–331.
Rathke, E. and M. Jones (1974) Serum cerebrosides in multiple sclerosis, *J. Neurochem.*, 22: 311–313.
Ryberg, B. (1976) Complement-fixing antibrain antibodies in multiple sclerosis, *Acta neurol. scand.*, 54: 1–12.
Samuelsson, K. (1971) Separation and identification of cerebrosides in cerebrospinal fluid by gas chromatography — mass spectrometry, *Scand. J. Clin. Lab. Invest.*, 27: 382–391.
Sato, J. and I. Hara (1972) Anti-cholesterol activity in antisera against human serum lipoprotein, *Immunochemistry*, 9: 585–587.
Schmid, G., G. Thomas, D. Hempel and W. Grüninger (1974) Radioimmunologic determination of myelin basic protein (MBP) and MBP-antibodies, *Europ. Neurol.*, 12: 173–185.
Schumacher, G. A., G. Beebe, R. F. Kibler, L. T. Kurland, J. F. Kurtzke, F. McDowell, B. Nagler, W. A. Sibley, W. W. Tourtellotte and T. L. Willmon (1965) Problems of experimental trials of therapy in multiple sclerosis — Report of the panel on the evaluation of experimental trials of therapy in multiple sclerosis, *Ann. N.Y. Acad. Sci.*, 122: 552–561.
Seil, F. J., M. E. Smith, A. L. Leiman and J. M. Kelly III (1975) Myelination inhibiting and neuroelectric blocking factors in experimental allergic encephalomyelitis, *Science*, 187: 951–953.
Suzuki, K. and Y. Suzuki (1972) Galactosyl ceramide lipidosis—Globoid cell leucodystrophy (Krabbe's disease). In: J. B. Stanbury, J. B. Wyngarden and D. S. Fredriksson (Eds.), *The Metabolic Basis of Inherited Disease*, 3rd edition, McGraw-Hill Book Company, New York NY, pp. 760–782.
Taketomi, T. and T. Yamakawa (1963) Immunochemical studies of lipids, *J. Biochem. (Tokyo)*, 5: 444–451.
Teitelbaum, D., R. Arnon, M. Sela, Y. Rabinsohn and D. Shapiro (1973) Sphingomyelin specific antibodies elicited by synthetic conjugates, *Immunochemistry*, 10: 735–743.
Tourtellotte, W. W. and A. F. Haerer (1969) Lipids in cerebrospinal fluid, Part 12 (In multiple sclerosis and retrobulbar neuritis), *Arch. Neurol. (Chic.)*, 20: 605–615.
Tourtellotte, W. W. and J. A. Parker (1966) Multiple sclerosis — Correlation between immunoglobulin-G in cerebrospinal fluid and brain, *Science*, 154: 1044–1046.
Venable, J. H. and Coggshall, R. (1965) A simplified lead citrate stain for use in electron microscopy, *J. Cell Biol.*, 25: 407–408.
Volanakis, J. E. and M. H. Kaplan (1974) Interaction of C-reactive protein complexes with the complement system, Part 2 (Consumption of guinea pig complement by CRP complexes: requirement for human C 1q), *J. Immunol.*, 113: 9–17.
Weissman, G., A. Brand and E. C. Franklin (1974) Interaction of immunoglobulins with liposomes, *J. Clin. Invest.*, 53: 536–543.
Wieme, R. J. (1959) An improved technique of agar gel electrophoresis on microscopic slides, *Clin. chim. Acta*, 4: 317–321.
Woyciechowska, J. L. and W. J. Brzosko (1977) Immunofluorescence study of brain plaques from two patients with multiple sclerosis, *Neurology (Minneap.)*, 27: 620–622.
Yokoyama, M., E. G. Trams and R. O. Brady (1962) Sphingolipid antibodies in sera of animals and patients with central nervous system lesions, *Proc. Soc. exp. Biol. Med. (N.Y.)*, 111: 350–352.

Paper II

IMMUNOGLOBULIN CHARACTERIZATION BY BACTERIAL ABSORPTION OF ANTIBRAIN ANTIBODIES IN MULTIPLE SCLEROSIS

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ABSTRACT

Complement-fixing (CF) antibrain antibodies are frequently found in serum and CSF in multiple sclerosis (MS). They represent several specificities and appear to be synthesized on both sides of the blood-brain barrier. Five sera and six CSF samples from 11 MS patients, representing 6 different specificities of antibrain antibodies, were absorbed with a series of bacterial strains with affinity for various immunoglobulins. Reactivity with brain preparations was eliminated by absorption with the following IgG absorbents: Staphylococcus aureus strain Cowan I, group A streptococcus strain A R1, and group G streptococcus strain G 148, but not by absorption with strains with low or no affinity for IgG. The results indicate in all tested samples the IgG_1 and/or IgG_2 nature of the antibrain antibodies.

INTRODUCTION

Multiple sclerosis (MS) is frequently associated with a number of more or less disease-specific humoral factors of possible importance for the manifestations of the disease; several of them appear to be of immunoglobulin nature. Thus, antibodies to brain constituents have been reported in MS (Lumsden, 1972), but the findings are often difficult to interprete because of an unspecific affinity of normal human IgG to the CNS (Aarli et al., 1975; Lisak et al., 1975; Lumsden, 1975; Traugott et al., 1979; Kennedy and Lisak, 1979). Gliotoxic and myelinotoxic activities in vitro are often found in serum (Berg and Källén, 1961, 1962; Bornstein, 1963) and CSF (Lamoureux and Borduas, 1966) from MS patients. In vivo, MS CSF was myelinotoxic to tadpole optic nerve (Tabira et al., 1977a) probably by an IgG mediated mechanism (Tabira et al., 1977b). Further, factors with in vitro neuroelectric blocking properties and of probable IgG nature are frequently found in MS sera (Bornstein and Crain, 1965; Schauf and Davies, 1978). The importance and interrelation of these factors are still incompletely known.

The unspecific binding of normal IgG to CNS does not seem to influence the results obtained by the complement fixation (CF) technique. Thus, it has been shown that patients with MS and chronic myelopathy, but not those with other neurological disorders, have circulating complement-fixing (CF) antibrain antibodies in high frequency (Laurell and Link, 1972; Ryberg, 1976, 1978). The antibodies are directed against several autoantigens in the CNS (Ryberg, 1978) and they appear to be produced on both sides of the blood-brain barrier in greatly varying proportions (Ryberg, 1980). Chromatographical experiments indicate the IgG nature of the antibrain antibodies (Ryberg, 1976, 1978). To facilitate the characterization of these antibodies in a large number of individual MS samples, we used a different approach. The ability of a number of bacteria, with specific

surface receptors for various immunoglobulins (Kronvall et al., 1979), to absorb the antibrain antibodies was investigated.

MATERIALS AND METHODS

Serum and CSF

Five serum and six CSF samples with CF antibrain antibodies were obtained from 11 MS patients diagnosed by the criteria of Müller (1949) and Schumacher et al. (1965). The antibodies were known to react with one of the following antigens, of which only V and VI are definitively characterized (Ryberg, 1978): (I) Antigen associated with CNS myelin. Organ specific. Labile to heat. Not solubilized by saline or water. Nonlipid. Not species specific. (II) Antigen with the properties of antigen I except for species restriction (not found in bovine brain). (III) Antigen equally distributed between gray and white matter. Organ specific. Labile to heat. Not solubilized by saline. Nonlipid. (IV) Antigen present predominantly in gray matter. Labile to heat. Not solubilized by saline. Nonlipid. (V) Cerebroside (galactosylceramide). Relatively myelin specific. (VI) Sulfatide (galactosylceramide-3-sulfate). Relatively myelin specific.

All samples were free from disturbing anticomplementary activity. After centrifugation, the CSF specimens were heated to 56°C for 30 min simultaneously with the serum samples. All samples were stored at -20°C.

Antigen Preparations

White and gray matter of human brain without macroscopic disease involvement was obtained at autopsy less than 24 h after death. The tissue samples were stored at -100°C until used. The following homogenates were prepared by treatment at +4°C for 30 s with a Polytron ^{R} PT 10-35 (Kinematica GmbH, Luzern, Switzerland) at top speed: 0.1 g white matter in 10 ml saline, 0.2 g gray matter in 10 ml saline, and 0.1 g white matter + 0.2 g gray matter in 10 ml saline. These homogenates, which had very low anticomplementary activity, were used fresh as antigen preparations. White matter homogenate was used to test samples reacting with antigens preferentially found in this tissue (antigens I, II, V, and VI). Gray matter homogenate was used to test

a sample with antibodies to antigen IV and the gray + white matter homogenate was used for experiments with the sample with antibodies to antigen III.

Complement Fixation (CF) Test

The CSF test was performed on Plexiglass plates with U-shaped wells according to the micromethod of Fulton and Dumbell (1949). Five 50 % hemolysis units (MHD_{50}) of guinea pig complement were employed and a photometrically standardized 0.2 % suspension of sheep erythrocytes sensitized with 6 MHD_{50} of amboceptor served as hemolytic system. The reaction was carried out at $+4^{o}C$ overnight, and after the addition of sensitized sheep erythrocytes, the plates were incubated at $37^{o}C$ for 2 h before reading. 50 % or less hemolysis, visually estimated, was recorded as a positive reaction. Controls for anticomplementary activity were performed with antigen, serum, and CSF at twice the concentration used in the test. Veronal buffer (pH 7.2) containing 0.15 mmol/l Ca^{2+} and 0.5 mmol/l Mg^{2+} was used throughout the experiments.

Absorption Experiments

Absorptions were carried out with the following heat- and formaldehyde stabilized bacteria: Staphylococcus aureus strain Cowan I (NCTC No. 8530), group A streptococcus strains AR 1 and AW 43, group G streptococcus strain G 148, and Staphylococcus epidermidis strain L 603 (Kronvall et al., 1979). The immunoglobulin-binding characteristics of these strains are shown in table I. After repeated washings in veronal buffer the bacteria were pelleted by centrifugation, resuspended in aliquots of diluted serum of CSF, and incubated at room temperature for 1 h. The suspensions were then centrifuged at 2,000 g for 10 min in a swing out rotor, and the supernatants were recovered. In a typical experiment, the following pellet volumes were added to 3 ml aliquots of serum diluted 1:10 in veronal buffer. S. aureus Cowan I 0.3 ml, streptococcus strain AR 1 1.0 ml, strain AW 43 0.3 ml, strain G 148 1.0 ml, and S. epidermidis L 603 1.0 ml. Before further analysis the serum aliquots were concentrated to a 1:2 dilution of the original volume and the CSF aliquots were concentrated about 50 times. The concentrations were performed in a collodion bag (Sartorius, Membranfilter GmbH, Göttingen,

Table I. Immunoglobulin binding characteristics of the bacterial species employed in this study

	IgG_1	IgG_2	IgG_3	IgG_4	IgA	IgM
S. aureus Cowan I	+	+	-	+	(±)	(±)
Streptococcus AR 1	+	+	+	+	-	-
Streptococcus AW 43	(+)	(+)	(+)	(+)	+	-
Streptococcus G 148	+	+	+	+	-	-
S. epidermidis L 603	-	-	-	-	-	-

Table II. Reactivity of serum and CSF samples after bacterial absorptions

	Antibody specificity	Titer of antibrain antibody after bacterial absorptions				
		Cowan I	AR 1	AW 43	G 148	L 603
Serum						
1	I	1:<1	1:<1	1:4	1:<1	1:4
2	I	1:<1	1:<1	1:4	1:<1	1:16
3	II	1:<1	1:<1	1:2	1:<1	1:4
4	IV	1:<1	1:<1	1:8	1:<1	1:8
5	VI	1:<1	1:<1	1:4	1:<1	1:8
CSF						
6	I	1:<1	n.d.	n.d.	1:<1	1:8
7	I	1:<1	n.d.	n.d.	1:<1	1:4
8	I	1:<1	n.d.	n.d.	1:<1	1:4
9	II	1:<1	n.d.	n.d.	1:<1	1:8
10	III	1:<1	n.d.	n.d.	1:<1	1:4
11	V	1:<1	n.d.	n.d.	1:<1	1:4

n.d. = Not determined

FRG) as described by Mies (1953).

Quantitation of IgG

The concentration of IgG in samples subjected to bacterial absorptions was determined by single radial immunodiffusion (Mancini et al., 1965).

RESULTS

Reactivity with brain homogenates was eliminated in all serum and CSF samples by absorption with one or more of the following IgG absorbents: S. aureus Cowan I and streptococcus strains AR 1 and G 148. Compared to aliquots absorbed with S. epidermidis L 603 with no affinity for IgG, the reduction in antibody titers was significant (≥ 2 titer steps), and the decrease in IgG concentration was >90 %. Absorption with streptococcus strain AW 43, with low affinity for IgG, was not effective in eliminating the antibody. The results are presented in detail in table II.

DISCUSSION

S. aureus Cowan I containing protein A is a potent absorbent of human IgG subclasses 1, 2, and 4 (Kronvall and Williams, 1969) and of fractions of IgA and IgM (Harboe and Fölling, 1974). In addition to this bacterium, a number of bacterial species with recently discovered surface receptors for various immunoglobulins (Kronvall et al., 1979) were employed for the characterization of antibrain antibodies in this study. All serum and CSF samples, representing antibrain antibodies of 6 different specificities, were absorbed with S. aureus Cowan I, streptococcus strain G 148 (absorbing IgG of all 4 subclasses), and, as a control, S. epidermidis L 603, which lacks affinity for human immunoglobulins. Several samples were in addition absorbed with streptococcal strains AR 1 (absorbing IgG of all 4 subclasses) and AW 43 (absorbing IgA of both subclasses and to a minor degree, IgG).

The successful elimination of reactivity between MS samples

and normal human brain by absorption with streptococcal strains AR 1 and G 148 rule out the possibility that this reactivity is caused by immunoglobulin classes other than IgG. Of the IgG subclasses only 1, 2, and 3 are complement fixing (Spiegelberg, 1974). The efficient absorption of the antibrain antibodies by S. aureus Cowan I therefore indicates that they are usually of IgG_1 and/or IgG_2 subclass. IgG_1 is of particular interest in this context as this subclass usually gives the greatest contribution to the increase in IgG level in MS CSF (Palmer et al., 1976; Vandvik et al., 1976; Eikhoff et al., 1979).

Grundke-Iqbal and Bornstein (1979) were unable to eliminate an in vitro demyelinating factor in MS sera by absorption of IgG_1, IgG_2, and IgG_4 with protein A coupled to Sepharose. This factor therefore does not seem to be identical with the antibrain antibodies here studied.

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References

Aarli, J.A.; Aparicio, S.R.; Lumsden, C.E.; Tönder, O.: Binding of normal human IgG to myelin sheats, glia and neurons. Immunology 28:171-185 (1975).

Berg, O.; Källén, B.: An in vitro gliotoxic effect of serum from patients with multiple sclerosis and some other neurologic conditions. K. fysiogr. Sällsk. Lund Förh. 31:87-96 (1961).

Berg, O.; Källén, B.: Gliotoxic effect of serum from patients with neurological diseases. Lancet i:1051-1052 (1962).

Bornstein, M.B.: A tissue culture approach to demyelinate disorders. Nat. Cancer Inst. Monog. 11:197-214 (1963).

Bornstein, M.B.; Crain, S.M.: Functional studies of cultured brain tissues as related to 'demyelinative disorders'. Science 148:1242-1244 (1965).

Eickhoff, K.; Kaschka, W.; Skvaril, F.; Theilkaes, L.; Heipertz, R.: Determination of IgG subgroups in cerebrospinal fluid of multiple sclerosis patients and others. Acta neurol. scand. 60:277-282 (1979).

Fulton, F.; Dumbell, K.R.: The serological comparison of strains of influenza virus. J. gen. Microbiol. 3:97-111 (1949).

Grundke-Iqbal, J.; Bornstein, M.B.: Multiple sclerosis: immunochemical studies on the demyelinating serum factor. Brain Res. 160:489-503 (1979).

Harboe, M.; Fölling, I.: Recognition of two distinct groups of human IgM and IgA based on different binding to staphylococci. Scand. J. Immunol. 3:471-482 (1974).

Kennedy, P.G.E.; Lisak, R.P.: A search for antibodies against glial cells in the serum and cerebrospinal fluid of patients with multiple sclerosis and Guillain-Barré syndrome. J. neurol. Sci. 44:125-133 (1979).

Kronvall, G.; Simmons, A.; Myhre, E.B.; Jonsson, S.: Specific absorption of human serum albumin, immunoglobulin A, an immunoglobulin G with selected strains of group A and G streptococci. Infect. Immunity 25:1-10 (1979).

Kronvall, G.; Williams, R.C.: Differences in anti-protein A activity among IgG subgroups. J. Immun. 103:828-833 (1969).

Lamoreux, G.; Borduas, A.G.: Immune studies in multiple sclerosis. Clin. exp. Immunol. 1:363-376 (1966).

Laurell, A.-B.; Link, H.: Complement-fixing antibrain antibodies in multiple sclerosis. Acta neurol. scand. 48:461-466 (1972).

Lisak, R.P.; Zwiman, B.; Norman, M.: Antimyelin antibodies in neurologic diseases. Immunofluorescent demonstration. Archs Neurol., Chicago 32:163-167 (1975).

Lumsden, C.E.: The clinical immunology of multiple sclerosis; in McAlpine, Lumsden, Acheson, Multiple sclerosis. A reappraisal; 2nd ex., pp. 512-621 (Churchill/Livingstone, Edinburgh 1972).

Lumsden, C.E.: The nature and role of anti-myelin and 'antisynaptic' immune response in multiple sclerosis and experimental allergic encephalomyelitis; in Schuller, Immunopathologie du système nerveux. Colloque de synthèse, Hôpital de la Salpêtrière, Paris 1974 (Inserm, Paris 1975).

Mancini, G.; Carbonara, A.O.; Heremans, J.F.: Immunochemical quantification of antigens by single radial immunodiffusion. Immunochemistry 2:235-254 (1965).

Mies, H.-J.: Einengung von Liquor cerebrospinalis als Vorbereitung zur Papierelektrophorese. Klin. Wschr. 31:159-161 (1953).

Müller, R.: Studies on multiple sclerosis. Acta med. scand. 133: suppl. 222, pp. 1-214 (1949).

Palmer, D.L.; Minard, B.J.; Cawley, L.P.: IgG subgroups in cerebrospinal fluid in multiple sclerosis. New Engl. J. Med. 294: 447-448 (1976).

Ryberg, B.: Complement-fixing antibrain antibodies in multiple sclerosis. Acta neurol. scand. 57:1-12 (1976).

Ryberg, B.: Multiple specificities of antibrain antibodies in multiple sclerosis and chronic myelopathy. J. neurol. Sci. 38:357-382 (1978).

Ryberg, B.: Intrathecal and extrathecal production of antibrain antibodies in multiple sclerosis. J. neurol. Sci. 48:1-8 (1980).

Schauf, C.L.; Davis, F.A.: The occurrence, specificity, and role of neuroelectric blocking factors in multiple sclerosis. Neurology, Minneap. 28:34-39 (1978).

Schumacher, G.A.; Beebe, G.; Kibler, R.F.; Kurland, L.T.; Kurtzke, J.F.; McDowell, F.; Nader, B.; Sibley, W.A.; Tourtellotte, W.W.; Willmon, T.L.: Problems of experimental trials of therapy in multiple sclerosis. Report of the panel on the evaluation of experimental trials of therapy in multiple sclerosis. Ann. N.Y. Acad. Sci. 122:552-561 (1965).

Spiegelberg, H.L.: Biological activities of immunoglobulins of different classes and subclasses. Adv. Immunol. 19:259-293 (1974).

Tabira, T.; Webster, H. De F.; Wray, S.H.: In vivo test for myelinotoxicity of cerebrospinal fluid. Brain Res. 120:103-112 (1977a).

Tabira, T.; Wolfgram, F.J.; Webster, H.De F.; Wray, S.H.; McFarlin, D.E.: Myelinotoxicity of CSF fractions from multiple sclerosis patients tested in an in vivo model. Neurology. Minneap. 27:374 (1977b).

Traugott, V.; Snyder, S.; Raine, C.S.: Oligodendrocyte staining by multiple sclerosis serum is nonspecific. Ann Neurol. 6:13-20 (1979).

Vandvik, B.; Natvig, J.B.; Wiger, D.: IgG_1 subclass restriction of oligoclonal IgG from cerebrospinal fluids and brain extracts in patients with multiple sclerosis and subacute encephalitides. Scand. J. Immunol. 5:427-436 (1976).

Acta neurol. scandinav. *62*, 193–196, 1980 SHORT COMMUNICATION

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Multiple sclerosis: Search for a serologic inhibitor

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Complement-fixing (CF) antibrain antibodies are frequently found in serum and CSF in multiple sclerosis (MS). A negative result in the test for these antibodies migh depend not only on a lack of antibodies, but also on serologic inhibiting factors. To test this possibility, serum and concentrated CSF samples without demonstrable CF antibrain antibodies were allowed to interact with two standardized positive complement fixation reactions; one for antibrain antibodies and the other a Wasserman reaction. No evidence of inhibiting activity was found in 11 CSF and 15 serum samples from 20 MS patients. On the other hand, inhibiting activity was seen in serum from four of 12 patients with neoplasms and from three of 11 patients with rheumatoid arthritis.

Key words: Antibrain antibodies – malignant neoplasms – multiple sclerosis – rheumatoid arthritis – serologic inhibitor.

Complement-fixing (CF) IgG antibodies against several water-insoluble autoantigens in the CNS have been demonstrated in high frequency in patients with multiple sclerosis (MS) and chronic myelopathy as opposed to other neurological diseases (*Ryberg* 1976, 1978). Their distribution between CSF and serum indicates that they are produced on both sides of the blood-brain barrier in greatly varying proportions (*Ryberg* 1980).

A factor able to interfere with the antigen antibody reaction or complement fixation (CF) would complicate the evaluation of results obtained with the CF technique. Indeed, an inhibitor of the antigen antibody reaction has recently been reported in serum from patients with subacute sclerosing panencephalitis (*Karcher et al.* 1977). Rheumatoid factors (RF), sometimes found in MS sera (*Fraser et al.* 1979, *Salmi et al.* 1979), are well-known inhibitors of the CF reaction (*Heimer & Levin* 1965, *Riski et al.* 1977). Moreover, non-CF antibodies are thought to block CF antibodies in some systems (*Luster et al.* 1976), and circulating antigen fragments might well cause a specific inhibition of the CF (*Wasserman & Levine* 1961, *Whitaker et al.* 1975).

The present studies were designed to reveal serologic inhibitors that could lead to negative results in MS and control samples in tests for CF antibrain antibodies. A number of sera from patients with rheumatoid arthritis (RA) were included to demonstrate the ability of the methods to detect inhibiting factors. None of the MS samples exhibited any inhibitory activity, which was, however, unexpectedly found in a number of sera from patients with malignant neoplasms.

MATERIAL AND METHODS

Samples without demonstrable antibrain antibodies were selected mainly from an earlier study (*Ryberg* 1978). Thus, 11 CSF samples and 15 serum samples from 20 MS patients, CSF and serum from 22 patients with various neurological disorders

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other than MS, and sera from 11 RA patients were investigated. The study was extended to sera from eight patients with malignant neoplasms. All samples were heated to 56°C for 30 min and the CSF samples were concentrated by filtration in a collodion bag (Sartorius, Membranfilter GmbH, Göttingen, West Germany) as described by *Mies* (1953). IgG was quantitated by single radial immunodiffusion (*Mancini et al.* 1964). All samples were stored at –20°C.

White matter of human brain without macroscopic disease involvement was obtained at autopsy and stored at –20°C until used. Homogenate of white matter in 100 parts (w/v) cold saline was obtained by treatment for 30 sec with a Polytron® (Kinematica GmbH, Luzern, Switzerland) at top speed. The homogenate, which had very low anticomplementary activity, was used fresh as antigen preparation.

The CF test was performed on plexiglass plated with U-shaped wells according to the method of *Fulton & Dumbell* (1949) as previously described (*Ryberg* 1978). Two standardized indicator reactions were used to reveal inhibiting factors. In the first, a MS serum (serum 100 (*Ryberg* 1978)) known to react with a heat-labile nonlipid antigen in human CNS myelin (antigen I (*Ryberg* 1978)) was employed. In the other, a Wasserman positive serum was used. After initial dilution with veronal buffer to give a positive reaction in a titre of 1:2 against the corresponding antigen (white matter homogenate and a standardized Wasserman antigen, respectively) in the CF test, these two sera were titrated in the presence of the sample under investigation. Thus, one extra drop of CSF concentrated about 40 times or one drop of serum diluted 1:5 in veronal buffer was added to each reaction well. A reduction in the serum titre of the indicator reactions by at least one twofold titre step was taken as evidence of inhibitory activity in the added sample.

RESULTS

None of the MS samples caused an inhibition of the indicator reactions. On the contrary two MS CSF caused an enhancement by one twofold titre step in the antibrain antibody reaction, probably indicating the presence of antibrain antibodies in these samples.

None of the 22 control CSF samples and only five of 33 non-MS sera exerted a reproducible inhibition of the antibrain antibody reaction. The inhibiting sera were found in three of 11 tested RA patients and in two of four patients with neoplasms. Out of eight additional sera from patients with neoplasms, two inhibited the antibrain antibody reaction. Thus four of 12 sera from patients with neoplasms gave inhibition of the antibrain antibody reaction. The tumors associated with inhibiting serum were: carcinoma of the prostate, breast carcinoma, kidney carcinoma and an unspecified anaplastic cancer. All these tumors had given metastases. The tumors associated with noninhibiting serum were: osteosarcoma, chondrosarcoma, unspecified sarcoma, chordoma, malignant glioma, carcinoma of the prostate, and meningioma (two patients). Two of them had known metastases.

Inhibition of the Wasserman reaction was caused by only one sample, an RA sample, that also inhibited the antibrain antibody reaction. Evidently the latter indicator reaction was more sensitive.

DISCUSSION

The tests for serologic inhibitors were designed to detect unspecific inhibition of the reaction between antibody and antigen, and factors influencing the CF test. They

were performed under conditions similar to those presently used in experiments to detect CF antibrain antibodies. MS serum rich in the CF autoantibody most prevalent in MS patients was selected for one of the indicator reactions to make possible also the detection of a more specific inhibitor. Although no MS sample showed any inhibiting activity, the findings with some non-MS sera demonstrate the ability of the tests to detect inhibition. The antibrain antibody indicator reaction might conceivably fail to detect inhibiting factors if a sample also contains antibrain antibody. The simultaneous testing in a different antigen antibody system, such as the Wasserman reaction, was therefore important.

The finding in sera from patients with various malignant disseminated neoplasms of activity inhibiting the CF test for antibrain antibodies is interesting, but needs confirmation as the present experiments were designed merely to reveal evidence of inhibitors, not to conclusively demonstrate their existence.

In summary, failure to detect antibrain antibodies in the CF test does not appear to depend on inhibiting factors in the patient groups of greatest interest for such a study.

REFERENCES

Fraser, K. B., M. Armstrong, P. V. Shirodaria & M. Gharpure (1979): Affinity for measles virus anti-haemolysin of residual immunoglobulin M in sera of some patients with multiple sclerosis. Clin. Exp. Immunol. *36,* 304–310.

Fulton, F. & K. R. Dumbell (1949): The serological comparison of strains of influenza virus. J. Gen. Microbiol. *3,* 97–111.

Heimer, R. & F. Levin (1965): Inhibition of complement fixation by rheumatoid sera. Ann. N.Y. Acad. Sci. *124* (II), 879–883.

Karcher, D., M. Noppe & A. Lowenthal (1977): A heat stable serum inhibitor of an antigen antibody reaction of subacute sclerosing panencephalitis. J. Neurol. *216,* 51–56.

Luster, M. J., R. C. Armen, J. V. Hallum & G. A. Leslie (1976): Measles virus-specific IgG antibodies in patients with subacute sclerosing panencephalitis. Proc. Nat. Acad. Sci. (USA) *73,* 1297–1299.

Mancini, G., A. O. Carbonara & J. F. Heremans (1965): Immunochemical quantification of antigens by single radial immunodiffusion. Immunochemistry *2,* 235–254.

Mies, H.-J. (1953): Einengung von Liquor cerebrospinalis als Vorbereitung zur Papier-Elektrophorese. Klin. Wschr. *31,* 159–161.

Riski, H., S. Pyrhönen, O. Wager & K. Penttinen (1977): Lack of measurable complement fixing antibodies against virus antigens. Acta Path. Microbiol. Scand., Sect. B, *85,* 167–173.

Ryberg, B. (1976): Complement-fixing antibrain antibodies in multiple sclerosis. Acta Neurol. Scand. *54,* 1–12.

Ryberg, B. (1978): Multiple specificities of antibrain antibodies in multiple sclerosis and chronic myelopathy. J. Neurol. Sci. *38,* 357–382.

Ryberg, B. (1980): Intrathecal and extrathecal production of antibrain antibodies in multiple sclerosis. J. Neurol. Sci. In press.

Salmi, A., B. R. Ziola, T. Vuorimaa, T. Arnadottir & M. Reunanen (1979): Measles IgM antibodies and IgM anti-IgG activity in subacute sclerosing panencephalitis (SSPE) and multiple sclerosis (MS). Irish J. Med. Sci. *147,* 121.

Wasserman, E. & L. Levine (1961): Quantitative micro-complement fixation and its use in the study of antigenic structure by specific antigen-antibody inhibition. J. Immunol. *87,* 290–295.

Whitaker, J. N., C.-H. J. Chou, F. C.-H. Chou & R. F. Kibler (1975): Antigenic determinants of bovine myelin encephalitogenic protein recognized by rabbit antibody to myelin encephalitogenic protein. J. Biol. Chem. *250*, 9106–9111.

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INTRATHECAL AND EXTRATHECAL PRODUCTION OF ANTIBRAIN ANTIBODIES IN MULTIPLE SCLEROSIS

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SUMMARY

Complement-fixing antibrain antibodies of several specificities were titrated in paired serum and CSF samples from 27 MS patients. The results indicated that the antibodies were synthesized on both sides of the blood–brain barrier in proportions that showed a great interindividual variation. It is suggested that this variation applies also to other MS-associated immunoglobulins.

INTRODUCTION

Multiple sclerosis (MS), a disease of unknown etiology, is frequently associated with autoimmune phenomena. Of these phenomena it has been suggested that the antibrain antibodies, reviewed by Lumsden (1972), play an important role in the demyelinating process (Lumsden 1975). The study of these antibodies has been hampered by the nonspecific affinity of normal human IgG to components in the central nervous system (Aarli et al. 1975; Lisak et al. 1975; Lumsden 1975). This difficulty, however, does not seem to influence significantly the results obtained by the complement fixation (CF) technique. Thus, complement-fixing (CF) IgG antibodies against various water-insoluble autoantigens in the CNS have been demonstrated in high frequency in patients with MS and chronic myelopathy as opposed to other neurological diseases (Ryberg 1976, 1978). The antibodies were usually demonstrable in CSF, but not in serum at a standardized IgG concentration of 1000 mg/l. When antibrain antibodies were present in both fluids, the antibody level related to IgG concentration appeared to be higher in CSF. As knowledge of the

Abbreviations: CSF-antibody and serum-antibody denote the reciprocal antibody titres in the two fluids.

distribution of the antibodies between the two compartments might provide information on their origin, an investigation of MS from this aspect was deemed of interest.

MATERIAL AND METHODS

Paired samples of serum and lumbar CSF were obtained from 27 MS patients diagnosed by the criteria of Müller (1949) and of Schumacher et al. (1965). All were known to have CF antibrain antibodies in serum and/or CSF. In 12 of the patients the antibrain antibodies had been demonstrated earlier to react with one of the following antigens, of which only V and VI are definitively characterized (Ryberg 1978):

I. Antigen associated with CNS myelin. Organ specific. Labile to heat. Not solubilized by saline or water. Nonlipid. Not species specific.
II. Antigen with the properties of antigen I except for species restriction (not found in bovine brain).
III. Antigen equally distributed between grey and white matter. Organ specific. Labile to heat. Not solubilized by saline. Nonlipid.
IV. Antigen present predominantly in grey matter and possibly present in some nonneural tissues. Labile to heat. Not solubilized by saline. Nonlipid.
V. Cerebroside (galactosylceramide). Relatively myelin specific.
VI. Sulfatide (galactosylceramide-3-sulfate). Relatively myelin specific.

After cell counting and centrifugation, the CSF specimens were heated to 56 °C for 30 min simultaneously with the serum samples and were concentrated about 100 times by ultrafiltration in a collodion bag (Sartorius, Membranfilter GmbH, Göttingen, F.R.G.) as described by Mies (1953). IgG was quantitated in unconcentrated CSF, in concentrated CSF, and in serum by single radial immunodiffusion (Mancini et al. 1964). Albumin was similarly quantitated in unconcentrated CSF and in serum. CSF and serum from each patient were analysed on the same plate. All samples were stored at −20 °C. Before testing in the complement fixation test, serum was diluted in veronal buffer in serial 2-fold steps (neat – 1 : 64), and to cope with the low titres, concentrated CSF was diluted in veronal buffer to give IgG concentrations 0.5, 1, 2, 4, 8 and 16 times the IgG concentration of the corresponding unconcentrated CSF sample.

White and grey matter of human brain without macroscopic disease involvement was obtained at autopsy less than 24 h after death. The tissue samples were stored at −20 °C until used. Homogenates of white and grey matter in 100 parts (w/v) cold saline were obtained by treatment for 30 s with a Polytron® (Kinematica GmbH, Luzern, Switzerland) at top speed. The homogenates, which had very low anticomplementary activity, were used fresh as antigen preparations. The grey matter homogenate was used only to test samples that from earlier studies were known to give stronger reactions with this antigen than with white matter homogenate.

The complement fixation test was performed according to the method of Fulton and Dumbell (1949) as previously described (Ryberg 1978). Samples free from disturbing anticomplementary activity, as seen in control experiments performed by the same technique, were selected for this study.

RESULTS

There was a wide range of titre values in CSF and serum and of the ratios between these values in paired samples (Table 1 and Fig. 1). No linear relation

TABLE 1

RESULTS OF IgG, ALBUMIN, AND ANTIBRAIN ANTIBODY DETERMINATIONS

					Antibrain antibody		
Patient number	CSF-IgG (g/l)	Serum-IgG (g/l)	CSF-albumin (g/l)	Serum-albumin (g/l)	CSF-titre	Serum-titre	Specificity
1	0.048	6.4	0.32	42	1 : $\frac{1}{2}$	1 : < 1	I
2	0.067	11.4	0.17	35	1 : $\frac{1}{4}$	1 : 2	I
3	0.038	7.6	0.17	49	1 : $\frac{1}{8}$	1 : < 1	I
4	0.033	9.8	0.14	47	1 : $\frac{1}{4}$	1 : 32	I
5	0.143	7.6	0.22	36	1 : $\frac{1}{4}$	1 : 8	I
6	0.073	7.3	0.32	40	1 : 1	1 : 16	I
7	0.190	8.3	0.30	38	1 : $\frac{1}{4}$	1 : 4	I
8	0.087	11.1	0.26	41	1 : $\frac{1}{8}$	1 : < 1	II
9	0.051	10.1	0.13	38	1 : $\frac{1}{8}$	1 : 2	III
10	0.043	7.4	0.23	46	1 : $\frac{1}{8}$	1 : 8	IV
11	0.050	10.0	0.11	34	1 : $\frac{1}{8}$	1 : < 1	V
12	0.077	7.3	0.27	48	1 : $\frac{1}{4}$	1 : 32	VI
13	0.074	7.9	0.19	44	1 : 1	1 : 4	n.d.
14	0.083	11.0	0.11	36	1 : $\frac{1}{16}$	1 : < 1	n.d.
15	0.094	9.9	0.19	38	1 : $\frac{1}{16}$	1 : < 1	n.d.
16	0.057	9.1	0.19	39	1 : $\frac{1}{4}$	1 : 1	n.d.
17	0.054	10.6	0.16	48	1 : $\frac{1}{8}$	1 : < 1	n.d.
18	0.108	11.0	0.22	37	1 : $\frac{1}{2}$	1 : 2	n.d.
19	0.094	10.5	0.15	35	1 : $\frac{1}{8}$	1 : < 1	n.d.
20	0.036	10.1	0.18	41	1 : $\frac{1}{16}$	1 : < 1	n.d.
21	0.099	11.3	0.22	38	1 : $\frac{1}{4}$	1 : 8	n.d.
22	0.069	11.1	0.15	37	1 : $\frac{1}{16}$	1 : 4	n.d.
23	0.209	10.9	0.15	49	1 : $\frac{1}{4}$	1 : 2	n.d.
24	0.038	8.1	0.19	37	1 : $\frac{1}{4}$	1 : 2	n.d.
25	0.052	9.4	0.21	36	1 : $\frac{1}{16}$	1 : < 1	n.d.
26	0.078	12.2	0.21	36	1 : $\frac{1}{16}$	1 : < 1	n.d.
27	0.044	8.3	0.21	40	1 : < $\frac{1}{16}$	1 : 4	n.d.

n.d. = not determined.

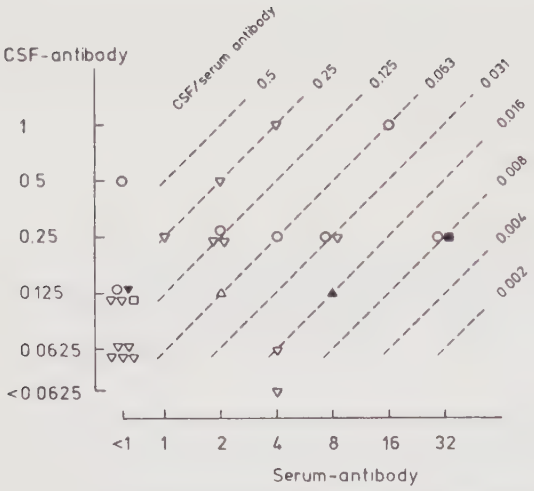

Fig. 1. The relation between CF antibrain antibody titres in CSF and serum in the 27 MS patients. CSF-antibody and serum-antibody denote the reciprocal antibody titres in the two fluids. The diagonal lines give the CSF/serum antibody ratios for paired CSF and serum observations. Patients with characterized antibody (Ryberg 1978) are denoted as follows: ○ = specificity I, □ = specificity II, △ = specificity III, ▲ = specificity IV, ▼ = specificity V, and ■ = specificity VI. ▽ denotes patients with uncharacterized antibrain antibodies.

between titres in CSF and serum existed, but CSF antibody levels $\geqslant 0.25$ were significantly more frequent in patients with demonstrable antibrain antibodies in serum than in patients without this property ($P < 0.001$ by Fisher's exact test).

INTERPRETATION

Most CSF/serum antibody ratios were higher than known ratios for a number of reference antibodies (Norrby et al. 1974; Fossan 1977), the normal CSF/serum IgG ratio (0.002–0.003), and the CSF/serum IgG ratios in the individual patients (Fig. 2).

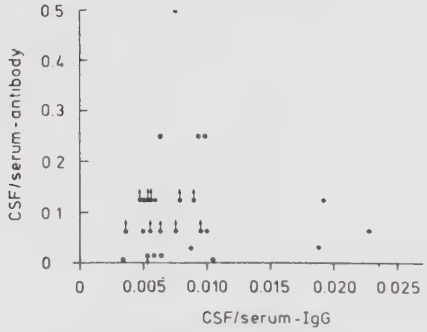

Fig. 2. The relation between CSF/serum antibody and CSF/serum IgG. In several patients the antibody level in CSF or serum was below the level of detection. In these cases minimum or maximum values of the ratios are calculated from the present data, and the fact that the actual value may be higher or lower is indicated by an arrow.

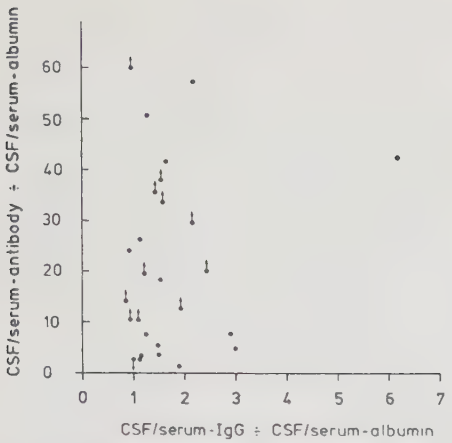

Fig. 3. The relation between CSF/serum antibody ÷ CSF/serum albumin and CSF/serum IgG ÷ CSF/serum albumin. For explanations, see Fig. 2.

If the ratio CSF/serum antibody ÷ CSF/serum albumin is formed in analogy to the ratio CSF/serum IgG ÷ CSF/serum albumin (Field 1954; Delpech and Lichtblau 1972; Ganrot and Laurell 1974; Tibbling et al. 1977) to compensate for a possible impairment of the blood–brain barrier, the present material shows a very wide range of values, most of them being higher than the corresponding CSF/serum IgG ÷ CSF/serum albumin ratios (Fig. 3).

The following calculation is an attempt to find a somewhat more elaborate relation between the CSF/serum ratio for IgG and the proportion of it that is synthesized intrathecally. It is based on some uncertain data and should be used cautiously until further experience is gained: Let a stand for the number of units of IgG produced per day and let x stand for the percentage of these units produced intrathecally. Essentially, all CSF IgG will be rapidly transferred by bulk flow (Lippincott et al. 1965; Hochwald and Wallenstein 1967; Cutler et al. 1970b) to the extrathecal space where it will distribute in plasma and interstitial fluid together with extrathecally synthesized IgG. At steady state, the synthesis of a IgG units/day is equal to the fractional turnover rate of IgG (FTR) × the plasma pool of IgG (Andersen 1964). The plasma pool is then $\frac{a}{\text{FTR}}$ units. With a plasma volume of about 3 l, the plasma concentration will be about $\frac{a}{3000 \cdot \text{FTR}}$ units/ml. Assuming a daily CSF production of 500 ml (Tourtellotte 1975), the IgG concentration of the CSF leaving the intrathecal space through the arachnoidal villi (cortical subarachnoidal fluid) can be calculated to be $\frac{a \cdot x}{500 \cdot 100}$ units/ml derived from direct intrathecal synthesis, plus a contribution from plasma, i.e. recirculating and extrathecally synthesized IgG. The size of this contribution depends on the state of the blood–brain barrier and can be calculated to result in an elevation of the cortical subarachnoidal fluid concentration of

$$\frac{a}{3000 \cdot \text{FTR}} \cdot 0.5 \cdot \frac{\text{CSF-albumin}}{\text{serum-albumin}} \cdot 1.7 \text{ units/ml}$$

as the CSF/serum ratio for albumin is about twice the CSF/serum ratio for IgG in patients with a normal or slightly impaired blood–brain barrier and without significant intrathecal IgG synthesis (Delpech and Lichtblau 1972; Ganrot and Laurell 1974; Tibbling et al. 1977). The figure 1.7 is an approximation derived from the observation that the total protein concentration in cortical subarachnoidal fluid is 1.5–2 times that of lumbar CSF (Cutler et al. 1970a; Oldendorf 1972). Thus

$$\frac{\text{CSF-IgG}}{\text{serum-IgG}} \approx \frac{\frac{1}{1.7} \cdot \left(\frac{a \cdot x}{500 \cdot 100} + \frac{a}{3000 \cdot \text{FTR}} \cdot 0.5 \frac{\text{CSF-albumin}}{\text{serum-albumin}} \cdot 1.7 \right)}{\frac{a}{3000 \cdot \text{FTR}}} =$$

$$= 0.035 \cdot \text{FTR} \cdot x + 0.5 \cdot \frac{\text{CSF-albumin}}{\text{serum-albumin}}$$

or

$$x \approx \frac{28 \cdot \text{CSF-IgG}}{\text{FTR} \cdot \text{serum-IgG}} - \frac{14 \cdot \text{CSF-albumin}}{\text{FTR} \cdot \text{serum-albumin}}$$

This formula applied to the present antibody data indicates that the CSF/serum albumin ratios were so low (mean 0.005; range 0.0030–0.0081) that the contribution to the CSF/serum antibody ratios from antibody leakage through the blood–brain barrier is probably insignificant in most cases (mean 0.0025; range 0.0015–0.004). Cytological data ruled out a significant contribution by bleeding into the CSF. Assuming for these IgG antibodies a FTR of a fairly uniform level, not too far from that of IgG in normal subjects (i.e. about 0.07), the wide range of values of the CSF/serum antibody ratio therefore probably indicates that the antibrain antibodies are in greatly varying proportions synthesized in the extrathecal and intrathecal compartments. The results presented in Figs. 2 and 3 support this conclusion.

DISCUSSION

The wide range of CSF/serum ratios for antibrain antibodies in this material of 27 MS patients probably means that the antibrain antibodies are synthesized in greatly varying proportions in the intrathecal and extrathecal compartments, as could be inferred from several lines of evidence.

Factors able to inhibit the antigen antibody reaction (Karcher et al. 1977) or CF (Heimer and Levin 1965) would complicate the evaluation of the present antibody data. No evidence of such inhibiting activity was found, however, in several MS samples investigated (to be published separately). A reduced CSF production, which might disturb the calculations, was improbable here, as seen from the fairly

normal CSF/serum albumin ratios. The intrathecal synthesis of IgG in patients with MS is well documented (Frick and Scheid-Seydel 1958; Lippincott et al. 1965; Cohen and Bannister 1967; Delpech and Lichtblau 1972; Ganrot and Laurell 1974; Sandberg-Wollheim 1974; Link and Tibbling 1977). As the present study indicates both an intrathecal and extrathecal synthesis of antibrain antibodies in MS patients, it is reasonable to speculate that this is true for MS-associated immunoglobulins in general. The varying amount of oligoclonal IgG in different MS CSF samples might thus depend not only on the absolute amount of oligoclonal IgG synthesized, but also on the proportion released into the CSF.

The size of the present material does not allow any conclusions on the relations between the antibody titres and clinical features. It should just be mentioned here that there was a tendency for higher CSF-IgG, CSF/serum IgG, CSF/serum IgG ÷ CSF/serum albumin, and greater disability in patients with higher CSF-antibody level. These and other relations are now being studied in a large number of MS patients.

Evidence has been presented of an intrathecal synthesis of antibodies against various antigens in some MS patients; namely, a number of viral antigens (Norrby 1978) RNA and DNA (Schuller et al. 1978), and smooth muscle (Nordahl and Vandvik 1977). This list is lengthened here by antibodies to several brain antigens. The intrathecal antibody synthesis, however, is not a general phenomenon in MS, and it can therefore probably not be explained by only a non-specific activation of immunoglobulin-producing cells.

REFERENCES

Aarli, J. A., S. R. Aparicio, C. E. Lumsden and O. Tönder (1975) Binding of normal human IgG to myelin sheats, glia and neurons, *Immunology*, 28: 171–185.

Andersen, S. B. (1964) *Metabolism of Human Gamma Globulin*, Blackwell, Oxford.

Cohen, S. and R. Bannister (1967) Immunoglobulin synthesis within the central nervous system in disseminated sclerosis, *Lancet*, i: 366–367.

Cutler, R. W. P., J. E. Murray and L. R. Cornick (1970a) Variations in protein permeability in different regions of the cerebrospinal fluid, *Exp. Neurol.*, 28: 257–265.

Cutler, R. W. P., G. V. Watters and J. P. Hammerstad (1970b) The origin and turnover rates of cerebrospinal fluid albumin and gamma-globulin in man, *J. neurol. Sci.*, 10: 259–268.

Delpech, B. and E. Lichtblau (1972) Étude quantitative des immunoglobulines G et de l'albumine du liquide céphalo-rachidien, *Clin. chim. Acta*, 37: 15–23.

Field, E. J. (1954) The production of gamma-globulin in the central nervous system, *J. Neurol. Neurosurg. Psychiat.*, 17: 228–232.

Fossan, G. O. (1977) The transfer of IgG from serum to CSF, evaluated by means of a naturally occurring antibody, *Europ. Neurol.*, 15: 231–236.

Frick, E. and L. Scheid-Seydel (1958) Untersuchungen mit J^{131}-markiertem γ-Globulin zur Frage der Abstammung der Liquoreiweisskörper, *Klin. Wschr.*, 36: 857–863.

Fulton, F. and K. R. Dumbell (1949) The serological comparison of strains of influenza virus, *J. gen. Microbiol.*, 3: 97–111.

Ganrot, K. and C.-B. Laurell (1974) Measurement of IgG and albumin content of cerebrospinal fluid, and its interpretation, *Clin. Chem.*, 20: 571–573.

Heimer, R. and F. Levin (1965) Inhibition of complement fixation by rheumatoid sera, *Ann. N.Y. Acad. Sci.*, 124 (II): 879–883.

Hochwald, G. M. and M. C. Wallenstein (1967) Exchange of γ-globulin between blood, cerebrospinal fluid and brain in the cat, *Exp. Neurol.*, 19: 115–126.

Karcher, D., M. Noppe and A. Lowenthal (1977) A heat stable serum inhibitor of an antigen antibody reaction of subacute sclerosing panencephalitis, *J. Neurol.*, 216: 51–56.

Link, H., and G. Tibbling (1977) Principles of albumin and IgG-analyses in neurological disorders, Part 3 (Evaluation of IgG synthesis within the central nervous system in multiple sclerosis), *Scand. J. clin. Lab. Invest.*, 37: 397–401.

Lippincott, S.W., S. Korman, L.C. Lax and C. Corcoran (1965) Transfer rates of gamma globulin between cerebrospinal fluid and blood plasma (Results obtained on a series of multiple sclerosis patients), *J. nucl. Med.*, 6: 632–644.

Lisak, R.P., B. Zwiman and M. Norman (1975) Antimyelin antibodies in neurologic diseases — Immunofluorescent demonstration, *Arch. Neurol. (Chic.)*, 32: 163–167.

Lumsden, C.E. (1972) The clinical immunology of multiple sclerosis. In: D. McAlpine, C.E. Lumsden and E.D. Acheson (Eds.), *Multiple Sclerosis — A Reappraisal*, 2nd edition, Churchill Livingstone, Edinburgh, London, pp. 512–621.

Lumsden, C.E. (1975) The nature and role of anti-myelin and "anti-synaptic" immune response in multiple sclerosis and experimental allergic encephalomyelitis. In: M.E. Schuller (Ed.), *Immunopathologie du Système Nerveux* (Colloque de synthèse, Hôpital de la Salpêtrière, Paris, 8 novembre 1974), Institut National de la Santé et de la Recherche Médicale, Paris, pp. 65–79.

Mancini, G., J.B. Vaerman, A.O. Carbonara and J.F. Heremans (1964) A single-radial-diffusion method for the immunological quantification of proteins. In: H. Peeters (Ed.), *11th Colloquium on Protides of the Biological Fluids*, Elsevier Publishing Company, Amsterdam, pp. 370–373.

Mies, H.-J. (1953) Einengung von Liquor cerebrospinalis als Vorbereitung zur Papierelektrophorese, *Klin. Wschr.*, 31: 159–161.

Müller, R. (1949) Studies on multiple sclerosis, *Acta med. scand.*, 133 (Suppl. 222): 1–214.

Nordahl, H.J., and B. Vandvik (1977) Evidence of local synthesis of smooth-muscle antibodies in the central nervous system in isolated cases of multiple sclerosis and chronic lymphocytic meningoencephalitis, *Scand. J. Immunol.*, 6: 327–334.

Norrby, E. (1978) Viral antibodies in multiple sclerosis, *Progr. med. Virol.*, 24: 1–39.

Norrby, E., H. Link, and J.-E. Olsson (1974) Measles virus antibodies in multiple sclerosis, *Arch. Neurol. (Chic.)*, 30: 285–292.

Oldendorf, W.H. (1972) Cerebrospinal fluid formation and circulation, *Progr. nucl. Med.*, 1: 336–358.

Ryberg, B. (1976) Complement-fixing antibrain antibodies in multiple sclerosis, *Acta neurol. scand.*, 54: 1–12.

Ryberg, B. (1978) Multiple specificities of antibrain antibodies in multiple sclerosis and chronic myelopathy, *J. neurol. Sci.*, 38: 357–382.

Sandberg-Wollheim, M. (1974) Immunoglobulin synthesis in vitro by cerebrospinal fluid cells in patients with multiple sclerosis. *Scand. J. Immunol.*, 3: 717–730.

Schumacher, G.A., G. Beebe, R.F. Kibler, L.T. Kurland, J.F. Kurtzke, F. McDowell, B. Nagler, W.A. Sibley, W.W. Tourtellotte and T.L. Willmon (1965) Problems of experimental trials of therapy in multiple sclerosis — Report of the panel on the evaluation of experimental trials of therapy in multiple sclerosis, *Ann. N.Y. Acad. Sci.*, 122: 552–561.

Schuller, E., N. Delasnerie and P. Lebon (1978) DNA and RNA antibodies in serum and CSF of multiple sclerosis and subacute sclerosing panencephalitis patients, *J. neurol. Sci.*, 37: 31–36.

Tibbling, G., H. Link and S. Öhman (1977) Principles of albumin and IgG analyses in neurological disorders, Part 1 (Establishment of reference values), *Scand. J. clin. Lab. Invest.*, 37: 385–390.

Tourtellotte, W.W. (1975) What is multiple sclerosis? Laboratory criteria for diagnosis. In: A.N. Davison, J.H. Humphrey, A.L. Liversedge, W.J. McDonald and J.S. Porterfield (Eds.), *Multiple Sclerosis Research*, Her Majesty's Stationery Office, London, pp. 9–26.

Wells, J.V. (1976) Metabolism of immunoglobulins. In: H.H. Fudenberg, D.P. Stites, J.L. Caldwell and J.V. Wells (Eds.), *Basic and Clinical Immunology*, Lange, Los Altos, pp. 195–203.

Paper V

ANTIBRAIN ANTIBODIES IN MULTIPLE SCLEROSIS. RELATION TO CLINICAL VARIABLES

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SUMMARY

IgG antibrain antibodies (ABA) of several specificities can be demonstrated in multiple sclerosis (MS) with the complement fixation technique. This technique seems to discriminate between IgG specifically and non-specifically bound to CNS preparations. Complement-fixing ABA were titrated in paired serum and CSF samples from 87 patients with clinically definitive MS, 15 patients with probable MS, 29 patients with other neurological diseases, and 13 "healthy" controls. In addition, sera from 55 non-MS patients were tested. In 40 % of the sera and 88 % of the CSF samples from patients with clinically definitive MS, ABA reacting with human brain homogenate were demonstrated. The corresponding figures for probable MS were 21 % and 73 %, and for the controls 11 % and 6 %. Two of nine sera from patients with the Guillain-Barré syndrome were strongly positive. There was a tendency for higher CSF ABA titres in younger MS patients and in those with an earlier onset of disease. ABA titres in serum and CSF were both correlated with a more malignant course. Irrespective of the mechanism of induction of ABA in MS - an excessive immunogenic stimulation and/or a defective immunoregulation - they are potentially pathogenic in several ways, e.g. (1) by direct antibody action, (2) by interaction with complement, (3) by antibody-dependent cell-mediated cytotoxicity, and (4) by interaction with phagocytic cells.

Of several correlations among the routine CSF variables, the finding of more pronounced abnormalities in male patients was notable.

INTRODUCTION

Numerous findings suggest a local hyperimmunization in the central nervous system (CNS) in multiple sclerosis (MS). Perivascular cuffs of lymphocytes, plasma cells, and macrophages are seen in brain tissue (Prineas 1979). IgG in CSF (Lowenthal 1964, Link 1967) and CNS (Link 1972; Mattson et al. 1980) is often of an oligoclonal nature, and there are indications of an intrathecal IgG production (Frick and Scheid-Seydel 1958; Delpech and Lichtblau 1972; Sandberg-Wollheim 1974). Despite no virus having yet proved of etiologic importance to MS, and in the absence of signs of active viral infection, slightly increased serum antibody titres to some, but not all, common viruses are frequently found in MS (Ross et al. 1965). These antibodies show a restricted clonal heterogeneity (Nordal et al. 1978) and are often found to be synthesized within the CNS (Norrby et al. 1974).

Experimental studies indicating a reduced suppressor cell activity in peripheral blood during certain phases of the disease (Arnason and Antel 1978; Reinherz et al. 1980) support the concept that these findings are caused by a deficient immunoregulation, but additional mechanisms leading to a local activation or attraction of lymphocyte or plasma cell clones in MS brain have been suggested (Esiri 1977; Vandvik et al. 1979; Cremer et al. 1980). Such mechanisms and perhaps an excessive immunogenic stimulation may explain the autoimmune phenomena in MS, some of which are potentially pathogenic (Lisak 1980).

In contrast to the situation in other neurological diseases studied, complement-fixing autoantibodies against several water-insoluble brain constituents are demonstrable in high frequency in MS and chronic myelopathy (Ryberg 1976, 1978), whereas other autoantibodies have an ordinary prevalence (Wright et al. 1965; Symington et al. 1978; Nordal and Vandvik 1977; Ryberg 1978). These antibrain antibodies (ABA) belong to IgG subclasses 1 and/or 2 (Ryberg and Kronvall 1981). They appear to be produced on both

sides of the blood-brain barrier in proportions that show a great interindividual variation (Ryberg 1980), implying that the responsible MS-related immunological aberration may not be limited to the CNS, but often includes the extrathecal compartment.

The present study was performed to shed light on the relation between ABA and some clinical, cytological, and chemical variables in MS.

MATERIALS AND METHODS

Patients and sampling

Paired samples of CSF and serum were obtained from patients belonging to the following groups:

Clinically definitive MS. Eighty-seven unselected MS patients seen at our outpatient department and diagnosed according to the criteria of Müller (1949) and fulfilling the criteria of clinically definitive MS (McDonald and Halliday 1977). Many of these patients were also included in earlier studies (Ryberg 1976, 1978, 1980).

Probable MS. Fifteen patients fulfilling the criteria of probable MS (McDonald and Halliday 1977). Nine of them had MS of the early probable or latent type.

Other neurological diseases (OND). Twenty-nine patients with various neurological diseases and not considered to have MS (Table 1).

"Healthy" controls. Thirteen individuals investigated for minor complaints and found not likely to have organic disease.

The age and sex distribution in the four groups are displayed in Table 2. None of the samples was obtained during or within two months after immunosuppressive treatment.

Disability. The degree of disability at sampling was evaluated in patients with clinically definitive MS. Forty-eight patients with mild symptoms that to a slight extent or not at all influenced their professional and social life were classified as not disabled. Thirty-nine patients with symptoms that affected professional and social life to a considerable extent were classified as disabled (Olsson et al. 1976). Using retrospective data and in a few cases information obtained 1-2 years after sampling,

Table 1. Diagnoses of patients considered not to have multiple sclerosis

Diagnosis	Number of patients	
	Serum and CSF	Serum only
Amyotrophic lateral sclerosis	2	10
Parkinson´s disease	2	9
Huntington´s disease	1	
Cerebral infarction	5	9
Transient ischemic attack	2	
Malignant hemispheral glioma		1
Intramedullary tumours		4
Extramedullary tumours		6
Neurosyphilis	2	
Encephalitis		4
Lymphocytic meningo-radiculitis	1	
Herpes zoster		1
Guillain-Barré syndrome		9
Epilepsia	2	
Epilepsia + cerebellar atrophy	1	
Muscle disease	2	
Shoulder neuritis	1	
Polyneuropathy	2	
Mononeuropathy	1	
Traumatic myelopathy		1
Syringomyelia		1
Subacute combined degeneration of the spinal cord	1	
Vertigo	1	
Horton´s headache	3	
"Healthy"	13	

Table 2. Age and sex distribution

Age (yr)	Serum and CSF				Serum only
	MS (n=87)	MS? (n=15)	OND (n=29)	"Healthy" (n=13)	OND (n=55)
11-20	1	1			2
21-30	13	4	5	3	2
31-40	27	4	7	3	5
41-50	30	4	1	4	9
51-60	11	2	2	3	15
61-70	5		6		8
71-80			7		14
81-90			1		
Male/female	34/53	6/9	18/11	4/9	38/17

MS = Clinically definitive MS
MS?= Probable MS
OND= Other neurological diseases

the duration of disease before disability was reached was determined.

Course of disease. The categories of Källén et al. (1975) were used: 1 = repeated relapses, but complete or nearly complete remission and thus no persisting deficit. 2 = repeated relapses with neurological sequelae. 3 = steadily progressive course after initial relapses. Patients with a remittent course were subdivided according to clinical activity.

Clinical activity. A = no clinical activity of the disease from four weeks before to two weeks after sampling. B = exacerbation during this time.

Serum and CSF

After cell counting by phase contrast microscopy (Sörnäs 1967) and centrifugation, the CSF specimens were heated to 56°C for 30 min along with the serum samples. CSF was concentrated about 100 times by ultrafiltration in a collodion bag (Sartorius, Membranfilter GmbH, Göttingen, FRG) (Mies 1953). IgG was quantitated in unconcentrated CSF, in concentrated CSF, and in serum by

single radial immunodiffusion (Mancini et al. 1965). Albumin was similarly quantitated in unconcentrated CSF and in serum. All samples were stored at -20°C. Before testing in the complement fixation test, serum was diluted in veronal buffer in serial two-fold steps. To compensate for the low titres, concentrated CSF was diluted in veronal buffer to give IgG concentrations 0.25, 0.5, 1, 2, 4, 8, and 16 times the IgG concentration of the corresponding unconcentrated CSF sample.

The antisera to albumin and IgG (γ-chains) were purchased from Dako (Copenhagen, Denmark).

Antigen preparations

Tissue samples without macroscopic disease involvement were obtained at autopsy less than 24 hours after death from patients without disseminated neoplasm or infection and without systemic disease. The samples were stored at -100°C until further prepared.

Brain homogenate was obtained by treatment for 30 s of 0.1 g white matter + 0.2 g grey matter in 10 ml cold saline with a Polytron R PT 10-35 (Kinematica GmbH, Luzern, Switzerland) at top speed. The homogenate was used fresh as antigen preparation.

A cholesterol-enriched "total lipid antigen" (TLA) from brain was prepared with slight modification as described (Ryberg 1978). An amount of lipid extract (Folch-Pi 1972) from human brain corresponding to 0.5 mg dry substance was evaporated under nitrogen and 0.5 mg cholesterol (Serva Feinbiochemica, Heidelberg, FRG) was added. This material was dissolved in 0.1 ml ethanol and 0.9 ml of 0.9 % NaCl was added (Rapport and Graf 1967). Before testing, this antigen preparation was diluted 1:8 in 0.9 % NaCl.

Myelin was prepared from fresh human white matter by the method of Hallpike (1972). Purity was assayed by electron microscopy as described (Ryberg 1978).

Complement fixation test

The complement fixation test was performed on plexiglass plates with U-shaped wells according to the micromethod of Fulton and Dumbell (1949). Five 50 % haemolysis units (MHD_{50}) of guinea-pig complement were employed and a photometrically standardized 0.2 % suspension of sheep erythrocytes sensitized with 6 MHD_{50} of amboceptor served as haemolytic system. The reaction was

carried out at +4°C overnight, and after the addition of sensitized sheep erythrocytes, the plates were incubated at 37°C for two hours before reading. Fifty per cent or less haemolysis, visually estimated, was recorded as a positive reaction. Parallel to the test for ABA each sample was analysed for anticomplementary activity. This was performed by the same technique, omitting the antigen and adding two drops of each sample dilution. Because of slight anticomplementary activity in the antigen preparations ABA titres that were less than eight times higher than that of anticomplementary activity were discarded. Thus the lowest possible serum and CSF end points were 1:2 and 8:1, respectively. 'Titre' was here defined as the reciprocal of the end point dilution or concentration of serum and CSF giving a positive reaction. E.g., a CSF end point of 4:1 corresponds to a titre value of 0.25.

The inter-experimental variation of this assay was studied by testing a positive serum sample on five occasions in one month using two different batches of complement. With each antigen all end points fell within two titre steps.

STATISTICAL METHODS

The significance of differences in the routine laboratory variables was tested by Student´s t-test. Differences in titres were assessed using the Mann-Whitney U-test. Proportions were evaluated by Fisher´s exact test. Correlations were calculated using Pearson´s product-moment coefficients. Serum ABA titres showed a strongly non-normal distribution, and here Spearman´s correlation coefficients were also calculated with very similar results. Samples in which titres could not be determined because of anticomplementary activity were excluded from the calculations.

RESULTS

Characterization of the patients

In CSF and serum from 87 patients with clinically definitive MS, 15 patients with probable MS, 29 patients with other neurological diseases (Table 1), and 13 persons without evidence of

Table 3. Characterization of the patient groups
Only patients studied in both CSF and serum are included
Results are given as means ± SD.

	MS n = 87	MS? n = 15	OND n = 29	Healthy n = 13
Male/female	34/53	6/9	18/11	4/9
Age (yr)	41.2±10.3	39.2±11.5	53.2±19.3	40.8±9.6
Onset (yr)	28.8± 8.7	33.3± 8.2	—	—
Duration (yr)	12.4± 7.9	5.9± 8.6	—	—
CSF mononuclears[i]	8.1±12.1[a]	14.8±17.5[b]	3.2± 6.1	1.5±0.9
CSF-IgG (g/l)	0.074±0.047[c]	0.063±0.040[d]	0.035±0.022	0.023±0.005
Serum-IgG (g/l)	10.1± 2.4	9.2± 1.7	9.8± 2.5	11.0±2.6
CSF/serum albumin	4.6± 1.7	4.4± 1.4	6.2± 4.2	3.7±1.1
IgG-index	1.7± 0.9[c]	1.7± 1.2[e]	0.6± 0.1	0.6±0.1
IgG_{SYN}(mg/d)	23 ± 22[c]	19 ± 20[e]	1 ± 4	-2 ± 3
log_2 CSF ABA titre	-1.9± 1.3[f] (n = 78)	-2.3± 1.4[g] (n = 15)	-3.9± 0.6 (n = 26)	-4.0± 0.0 (n = 9)
log_2 serum ABA titre	1.1± 1.6[h] (n = 73)	0.4± 0.9 (n = 14)	0.5± 1.1 (n = 26)	0.0± 0.0 (n = 10)

MS = clinically definitive MS

MS? = probable MS

OND = other neurologic diseases

a Higher than OND, $P < 0.05$ (Student's t test)

b Higher than OND, $P < 0.01$, Healthy, $P < 0.05$ (Student's t test)

c Higher than OND, $P < 0.001$, and Healthy, $P < 0.001$ (Student's t test)

d Higher than OND, $P < 0.01$, and Healthy, $P < 0.01$ (Student's t test)

e Higher than OND, $P < 0.001$, and Healthy, $P < 0.01$ (Student's t test)

f Higher than OND, $P < 0.001$, and Healthy, $P < 0.001$ (Mann-Whitney U test)

g Higher than OND, $P < 0.01$, and Healthy, $P < 0.01$ (Mann-Whitney U test)

h Higher than Healthy, $P < 0.05$ (Mann-Whitney U test)

i Cells x 10^6/l

neurological disease the following cytological and chemical variables were determined: mononuclear cell count in CSF, CSF-IgG, serum-IgG, CSF-albumin, and serum-albumin. The CSF/serum albumin ratios were calculated to evaluate the state of the blood-brain barrier (Link and Tibbling 1977). The intrathecal IgG synthesis was studied by means of the IgG-index (Delpech and Lichtblau 1972; Tibbling et al. 1977) and by the formula of Tourtellotte (1975) (IgG_{SYN}). The results are summarized in Table 3. As expected, the mean values of CSF mononuclear cell count, CSF-IgG, IgG-index, and IgG_{SYN} were much higher in the MS groups than in the control groups, whereas mean serum-IgG was similar in the four groups. The CSF/serum albumin ratio was slightly above the age-dependent upper normal value (Link and Tibbling 1977) in about 9 % of the MS patients, and the mean value was higher ($0.05 < p < 0.1$) than in the healthy controls that had a similar age distribution.

Interest focused on patients with a clinically definitive MS and, unless otherwise stated, the results presented refer to this group of patients (Table 4). This material is more representative for patients seen at a neurological outpatient department than for the whole MS population. The severely disabled are underrepresented as are those with very mild symptoms.

A negative correlation existed between the age of patients and mononuclear cell counts in CSF, CSF-IgG, and IgG_{SYN}. Age at onset of MS was negatively correlated with CSF-IgG, serum-IgG, CSF/serum albumin ratio and IgG_{SYN}. Patients with a late age of onset of MS ($\geq$35 years) had comparatively low mononuclear cell counts in CSF. Duration of disease was negatively correlated with mononuclear cell counts in CSF (Tables 5 and 6). The different time variables are, however, significantly interrelated and the evaluation of these correlations is therefore problematic.

Some of the laboratory variables showed relatively strong interrelations (Table 6), and again temporal factors might have some influence. The female preponderance in this MS material agrees with most other materials (Acheson 1972). Male and female patients were comparable regarding age, age at onset of MS, duration of the disease, and the proportion of disabled (47 % vs. 43 %). However, there was a marked sex-related difference in CSF mononuclear cell count, CSF-IgG, CSF/serum albumin, and IgG_{SYN}, male patients having the higher values. Mean values and SD of

these variables were therefore calculated separately for male and female patients (Table 7). Although the differences did not always reach statistical significance it is notable that the means of CSF mononuclear cell count, CSF-IgG, IgG_{SYN}, and, with one exception based on only four observations, CSF/serum albumin ratio were higher in male patients, whether or not disabled, and in all types of clinical course and activity.

A significantly longer mean duration of MS was found in patients with more malignant courses ("2" and "3") than in those with a more benign course ("1") (Table 4). Those with a clinical course of type "1" had higher than average mononuclear cell counts in CSF, lower CSF-IgG values, and lower CSF/serum albumin ratios. Patients with a steady progression (type "3") had deviations in the opposite direction, whereas those with a course of type "2" were intermediate in these respects. The differences could partly be attributed to dissimilarities in age, age at onset, and duration of disease in the three groups (Table 4). Ten patients from each group that could be matched for sex and relatively closely for the temporal variables were therefore compared (Table 8). The differences that persisted best in this matched material were those in CSF-IgG and CSF/serum albumin ratio.

Patients in exacerbation ("B") were usually younger and had a lower age at onset of MS than those in remission ("A"), but were comparable with respect to serum and CSF variables. This similarity was found also in 12 pairs of patients matched for sex, duration of MS, age at onset, and age.

Mean age at onset and mean duration of MS were somewhat higher in disabled patients than in those not disabled (Table 4), and these groups therefore were not directly comparable. To avoid this difficulty, all patients reaching disability at various time intervals after onset (as judged from retrospective data and information obtained within two years after sampling) were compared with patients not disabled after a similar duration of MS (Table 9). The control patients were as closely as possible matched for sex, duration of disease, age at onset, and age. There was a tendency for the disabled patients to have higher levels of CSF-IgG and higher CSF/serum albumin ratios. In many instances, differences in mean CSF-IgG between the various groups could to a great extent be attributed to differences in the blood-brain

Table 4. Characterization of patients with clinically definitive MS according to sex, clinical course, disability, and clinical activity

Results are given as means ± SD.

	Male	Female	Clinical course			Disability		Clinical activity	
			1	2	3	+	-	A	B
	n=34 (39 %)	n=53 (61 %)	n=29 (33 %)	n=30 (34 %)	n=28 (32 %)	n=39 (45 %)	n=48 (55 %)	n=45 (52 %)	n=14 (16 %)
Male/female	34/0	0/53	8/21	11/19	15/13	16/23	18/30	15/30	4/10
Age (yr)	40.0±10.0	41.3±10.5	39.2±10.1	40.4±10.5	44.1±9.9	41.7±10.9	40.8± 9.8	41.3±10.5	35.2± 7.8
Onset (yr)	28.5± 7.0	29.1± 9.7	31.0± 8.1	27.0± 8.8	28.5±8.9	27.0± 9.0	30.3± 8.2	31.0± 8.2	22.7± 7.0
Duration (yr)	12.4± 8.4	12.3± 7.7	8.2± 7.2	13.6± 7.8 [a]	15.8±7.3 [b]	14.8± 7.8 [c]	10.4± 7.6	10.3± 7.7	12.3± 7.9
CSF-mononuclears [i]	12.4±17.0 [d]	5.4± 6.0	10.6±16.3	8.4±11.7	5.2±5.0	6.4±10.2	9.6±13.3	9.0±13.7	11.2±15.5
CSF-IgG (g/l)	0.092±0.063 [e]	0.061±0.028	0.068±0.062	0.072±0.035	0.081±0.040	0.074±0.034	0.073±0.056	0.070±0.055	0.069±0.030
Serum-IgG (g/l)	10.1± 2.2	10.1± 2.5	10.0± 2.0	10.1± 2.4	10.1±2.8	10.8± 2.6	9.3± 2.4	9.8± 2.2	10.8± 2.2
CSF/serum albumin	5.1± 2.0 [f]	4.3± 1.5	4.1± 1.2	4.5± 1.7	5.2±2.1 [g]	4.6± 1.9	4.3± 1.5	4.3± 1.5	4.3± 1.2
IgG-index	1.8± 1.1	1.6± 0.7	1.6± 1.1	1.7± 0.9	1.7±0.8	1.6± 0.7	1.8± 1.0	1.7± 1.1	1.6± 0.6
IgG_{SYN} (mg/d)	31. ± 30	17 ± 12	21 ± 29	22 ± 17	25 ± 17	22 ± 15	23 ± 26	22 ± 26	21 ± 14
log_2 CSF ABA titre	-1.8± 1.2 (n = 31)	-1.9± 1.4 (n = 47)	-2.1± 1.0 (n = 26)	-2.0± 1.4 (n = 28)	-1.5±1.4 (n = 24)	-1.5± 1.4 (n = 35)	-2.1± 1.2 (n = 43)	-2.1± 1.2 (n = 40)	-1.9± 1.2 (n = 14)
log_2 serum-ABA titre	1.0± 1.6 (n = 26)	1.1± 1.7 (n = 47)	0.4± 0.9 (n = 22)	1.2± 1.7 (n = 25)	1.5±1.9 [h] (n = 26)	1.4± 1.9 (n = 35)	0.7± 1.3 (n = 38)	1.0± 1.6 (n = 35)	0.4± 0.7 (n = 12)

a	Higher than clinical course 1,	$P \leq 0.01$	(Student's t test)
b	Higher than clinical course 1,	$P < 0.001$	(Student's t test)
c	Higher than non-disabled,	$P < 0.01$	(Student's t test)
d	Higher than female,	$P < 0.01$	(Student's t test)
e	Higher than female,	$P < 0.001$	(Student's t test)
f	Higher than female,	$P < 0.05$	(Student's t test)
g	Higher than clinical course 1,	$P < 0.05$	(Student's t test)
h	Higher than clinical course 1,	$P < 0.05$	(Mann-Whitney U test)
i	Cells x 10^6/l		

Table 5. Characterization of patients with clinically definitive MS according to age, age at onset of MS, and duration of disease.

Results are given as means ± SD

	Age (yr)			Onset (yr)			Duration (yr)		
	<35 n = 20	35-44 n = 37	≥ 45 n = 30	< 25 n = 32	25-34 n = 33	≥ 35 n = 22	< 7 n = 24	7-14 n = 34	≥ 15 n = 29
Male/female	9/11	16/21	9/21	13/19	14/19	7/15	12/12	11/23	11/18
Age (yr)	28.2± 3.5[a]	39.4± 2.9[a]	52.1±7.0[a]	34.8±7.4[h]	41.5± 9.5[i]	50.1±8.0	33.4±10.3[m]	40.0± 9.0[n]	48.2±9.3
Onset (yr)	21.4± 4.9[b]	27.7± 6.6[c]	35.2±8.5	20.3±3.8[a]	29.4± 3.1[a]	40.4±4.7[a]	30.5± 8.0	29.6±10.1	26.5±7.1
Duration (yr)	6.8± 4.2[d]	11.8± 6.2[e]	16.9±9.1	14.5±7.1[k]	12.0± 8.2	9.8±8.0	3.9± 1.8[a]	10.3± 2.4[a]	21.9±4.8[a]
CSF mononuclears[t]	14.9±14.8[f]	8.8±13.4[g]	2.8±2.2	8.3±8.5[l]	11.4±16.7[k]	2.9±4.0	16.2±19.9	5.6± 5.1[o]	4.5±3.5[o]
CSF-IgG (g/l)	0.091±0.065[g]	0.077± 0.045	0.058±0.029	0.093±0.053[l]	0.069±0.046	0.053±0.026	0.085±0.071	0.063±0.030	0.076±0.037
Serum-IgG (g/l)	10.4± 2.3	10.0± 2.4	10.0±2.5	11.0±2.5	9.5± 1.9	9.5±2.5	9.9± 2.1[p]	9.7± 2.3	10.6±2.7
CSF/serum albumin	5.1± 2.1	4.6± 1.5	4.2±1.7	5.1±1.8	4.3± 1.7	4.1±1.9	4.3± 1.6	4.8± 1.9	4.4±1.6
IgG-index	1.8± 1.1	1.7± 0.9	1.5±0.8	1.8±1.0	1.7± 0.9	1.5±0.8	1.9± 1.2	1.4± 0.5	1.8±0.9
IgG_{SYN} (mg/d)	30 ± 30[g]	24 ± 21	16 ± 14	30 ± 25[k]	21 ± 21	14 ± 12	29 ± 33	17 ± 12	23 ± 16
$\log_2$ CSF ABA titre	-1.5± 0.9[q] (n = 17)	-1.6± 1.4[q] (n = 32)	-2.3±1.3 (n = 29)	-1.5±1.3[r] (n = 27)	-1.9± 1.0 (n = 31)	-2.4±1.5 (n = 19)	-1.5± 1.2 (n = 20)	-2.1± 1.3 (n = 33)	-1.8±1.3 (n = 25)
$\log_2$ serum ABA titre	1.1± 1.6 (n = 17)	1.2± 1.7 (n = 31)	0.9±1.4 (n = 25)	1.1±1.8 (n = 28)	1.2± 1.6 (n = 27)	0.7±1.3 (n = 18)	1.1± 1.9 (n = 17)	0.9± 1.4 (n = 30)	1.3±1.7 (n = 26)

a Statistical significance not calculated

b Lower than age 35-44 years and $\geq$ 45 years, $P < 0.001$ (Student's t test)

c Lower than age $\geq$ 45 years, $P < 0.001$ (Student's t test)

d Lower than age 35-44 years, $P < 0.001$, and age $\geq$ 45 years, $P < 0.001$ (Student's t test)

e Lower than age $\geq$ 45 years, $P < 0.01$ (Student's t test)

f Higher than age $\geq$ 45 years, $P < 0.001$ (Student's t test)

g Higher than age $\geq$ 45 years, $P < 0.05$ (Student's t test)

h Lower than onset 25-34 years, $P < 0.01$, and $\geq$ 35 years, $P < 0.001$ (Student's t test)

i Lower than onset $\geq$ 35 years, $P < 0.001$ (Student's t test)

k Higher than onset $\geq$ 35 years, $P < 0.05$ (Student's t test)

l Higher than onset $\geq$ 35 years, $P < 0.01$ (Student's t test)

m Lower than duration $\geq$ 15 years, $P < 0.001$, and 7-14 years, $P < 0.05$ (Student's t test)

n Lower than duration $\geq$ 15 years, $P < 0.001$ (Student's t test)

o Lower than duration < 7 years, $P < 0.01$ (Student's t test)

p Higher than duration 7-14 years, $P < 0.05$ (Student's t test)

q Higher than age $\geq$ 45 years, $P < 0.05$ (Mann-Whitney U test)

r Higher than onset $\geq$ 35 years, $P < 0.05$ (Mann-Whitney U test)

s Cells x 10^6/l

Table 6. Correlation coefficients between the variables studied in the patients with clinically definitive MS

	Onset n = 87	Duration n = 87	CSF mono-nuclears n = 87	CSF-IgG n = 87	Serum-IgG n = 87	CSF/serum albumin n = 87	IgG-index n = 87	IgG_{SYN} n = 87	log_2 CSF ABA titre n = 78	log_2 serum ABA titre n = 73
Age	$+0.66^{XXX}$	$+0.27^{XX}$	-0.33^{XX}	-0.26^{X}	-0.12	-0.15	-0.10	-0.24^{X}	-0.20	-0.01
Onset		-0.24^{X}	-0.12	-0.30^{XX}	-0.31^{XX}	-0.26^{X}	-0.07	-0.23^{X}	-0.27^{X}	-0.14
Duration			-0.34^{XX}	-0.01	+0.15	+0.10	-0.05	-0.05	0.00	+0.15
CSF mononuclears				$+0.57^{XXX}$	-0.05	+0.06	$+0.54^{XXX}$	$+0.63^{XXX}$	+0.21	-0.06
CSF-IgG					$+0.24^{X}$	$+0.44^{XXX}$	$+0.72^{XXX}$	$+0.98^{XXX}$	$+0.23^{X}$	+0.07
Serum-IgG						-0.01	-0.09	0.00	+0.14	+0.13
CSF/serum albumin							-0.12	$+0.30^{XX}$	+0.16	+0.18
IgG-index								$+0.84^{XXX}$	+0.11	-0.11
IgG_{SYN}									+0.21	+0.02
log_2 CSF ABA titre										$+0.33^{XX}$ (n = 64)

Significance of difference from zero: $^{X}P < 0.05$, $^{XX}P < 0.01$, $^{XXX}P < 0.001$

Table 7. Characterization of male and female patients with clinically definitive MS

Results are given as means ± SD

MALE

	Clinical course			Disability		Clinical activity	
	1	2	3	+	—	A	B
	n=8 (24 %)	n=11 (32 %)	n=15 (44 %)	n=16 (47 %)	n=18 (53 %)	n=15 (44 %)	n=4 (12 %)
CSF mononuclears[f]	22.5±26.6[a]	12.7±17.1	6.7±5.3[c]	8.8±13.9	15.6±19.3[a]	14.4±20.9	26.2±24.5[a]
CSF-IgG (g/l)	0.112±0.105[a]	0.083±0.045	0.089±0.045	0.079±0.037	0.105±0.078[b]	0.096±0.083[a]	0.094±0.039
CSF/serum albumin	4.3± 0.9	4.6± 2.1	5.7±2.2	5.5± 2.3	4.7± 1.6	4.6± 1.8	4.1± 1.2
IgG-index	2.2± 1.8	1.9± 1.0	1.6±0.8	1.4± 0.5	2.2± 1.4[a]	2.0± 1.5	2.1± 0.5
IgG_{SYN} (mg/d)	42 ± 50[a]	27 ± 22	27 ± 20	22 ± 16	39 ± 37[b]	34 ± 40[a]	32 ± 17[a]
log_2 CSF ABA titre	1.9± 0.7 (n = 7)	-1.8± 1.3 (n = 10)	-1.7±1.3 (n = 14)	-1.5± 1.1 (n = 16)	-2.1± 1.2 (n = 15)	-1.9± 1.0 (n = 13)	-1.8± 1.5 (n = 4
log_2 serum ABA titre	0.8± 1.5 (n = 4)	0.8± 1.4 (n = 9)	1.2±1.8 (n = 13)	0.7± 1.3 (n = 13)	1.2± 1.7 (n = 13)	0.9± 1.5 (n = 10)	0.3± 0.6 (n = 3)

	FEMALE						
	n=21 (40 %)	n=19 (36 %)	n=13 (25 %)	n=23 (43 %)	n=30 (57 %)	n=30 (57 %)	n=10 (19 %)
CSF mononuclears[f]	6.4± 7.0	5.9± 6.3	3.5±4.0	4.7± 6.4	5.9± 5.8	6.3± 7.4	5.3± 2.4
CSF-IgG (g/l)	0.051± 0.021	0.066±0.027	0.072±0.034	0.072±0.031	0.053±0.022	0.058±0.027	0.060±0.021
CSF/serum albumin	4.0± 1.3	4.3±1.4	4.6±1.9	4.5± 1.5	4.1± 1.4	4.1± 1.4	4.3± 1.3
IgG-index	1.4± 0.5	1.6±0.8	1.8±0.8	1.7± 0.8	1.5± 0.6	1.5± 0.7	1.4± 0.6
IgG_{SYN} (mg/d)	13 ± 9	19 ± 12	22 ± 13	21 ± 14	14 ± 9	16 ± 12	16 ± 10
log_2 CSF ABA titre	-2.2± 1.1 (n = 19)	-2.1± 1.4 (n = 18)	-1.1±1.7 (n = 10)	-1.6± 1.6 (n = 19)	-2.1± 1.2 (n = 28)	-2.2± 1.3 (n = 27)	-2.0± 1.2 (n = 10)
log_2 serum ABA titre	0.3± 0.8 (n = 18)	1.5± 1.8[d] (n = 16)	1.9±2.0[e] (n = 13)	1.9± 2.0 (n = 22)	0.5± 0.9 (n = 25)	1.0± 1.6 (n = 25)	0.4± 0.7 (n = 9)

a Higher than corresponding female group, $P < 0.05$ (Student´s t test)

b Higher than corresponding female group, $P < 0.01$ (Student´s t test)

c Lower than 1, $P < 0.05$ (Student´s t test)

d Higher than 1, $P < 0.05$ (Mann-Whitney U test)

e Higher than non-disabled, $P < 0.05$ (Mann-Whitney U test)

f Cells x 10^6/l

Table 8. Characterization of patients with clinically definitive MS, that were matched for sex, duration of MS, onset of disease, and age. Separate matchings for Clinical course and Clinical activity were carried out.

Results are given as means ± SD

	Clinical course			Clinical activity	
	1 n = 10	2 n = 10	3 n = 10	A n = 12	B n = 12
Male/female	5/5	5/5	5/5	4/8	4/8
Age (yr)	43.8±12.5	45.9±11.4	47.7±11.0	35.8± 7.7	34.1± 7.7
Onset (yr)	30.7± 7.7	32.8± 9.6	34.5± 9.4	26.3± 6.3	23.9± 6.9
Duration (yr)	13.0± 9.6	13.1± 8.9	13.2± 8.9	9.5± 5.2	10.0± 5.7
CSF mononuclears[a]	8.2± 9.8	5.7± 5.7	5.8± 6.4	13.2±23.2	12.4±16.5
CSF-IgG (g/l)	0.054±0.021	0.063±0.029	0.078±0.038	0.096±0.084	0.068±0.032
Serum-IgG (g/l)	10.6± 2.4	9.4± 2.5	8.8± 2.6	10.9± 2.3	10.6± 2.3
CSF/serum albumin	3.9± 1.1[b]	3.9± 1.1[b]	5.0± 0.9	4.9± 1.5	4.4± 1.2
IgG-index	1.9± 1.4	1.8± 1.0	1.8± 0.6	1.9± 1.6	1.5± 0.5
IgG_{SYN} (mg/d)	27 ± 37	19 ± 14	26 ± 16	32 ± 42	20 ± 14
$\log_2$ CSF ABA titre	-2.2± 0.6[c] (n = 10)	-2.2± 1.3[c] (n = 9)	-0.9± 1.3 (n = 9)	-1.8± 1.2 (n = 11)	-2.0± 1.3 (n = 12)
$\log_2$ serum ABA titre	0.9± 1.5 (n = 7)	0.0± 0.0 (n = 6)	1.4± 1.8 (n = 9)	1.0± 1.6 (n = 10)	0.5± 0.7 (n = 11)

a Cells x 10^6/l

b Lower than clinical course 3, $P < 0.05$ (Student's t test, unpaired)

c Lower than clinical course 3, $P < 0.05$ (Mann-Whitney U test)

Table 9. Characterization of all patients with clinically definitive MS reaching 'disability' within various time intervals after onset of the disease and in patients not reaching disability within the same time. The latter patients were selected to match as closely as possible for sex, duration of MS, onset of disease, and age.

Results are given as means ± SD

	Disabled <5 years n = 10	Not disabled <5 years n = 10	Disabled after 5-10 years n = 14	Not disabled <10 years n = 14	Disabled after 10-15 years n = 8	Not disabled <15 years n = 8	Disabled after 15-20 years n = 8	Not disabled <20 years n = 8
Male/female	4/6	4/6	7/7	5/9	3/5	3/5	5/3	5/3
Age (yr)	38.2±11.2	39.8± 9.9	36.1±9.0	38.3±5.3	42.3±6.1	41.1±3.5	48.0±10.5	51.5±11.2
Onset (yr)	32.1±10.1	33.4± 9.3	24.4±9.3	25.7±5.8	25.3±6.0	23.0±4.9	25.8± 5.3	27.4± 9.4
Duration (yr)	6.1± 3.4	6.4± 1.8	11.6±3.0	12.5±3.6	16.9±4.7	18.3±2.3	22.8± 5.1	24.0± 4.5
CSF mononuclears[a]	13.5±18.6	7.9±10.2	5.3±4.9	8.3±4.7	6.9±5.6	6.4±2.1	3.9± 3.5	3.8± 2.8
CSF-IgG (g/l)	0.086± 0.042	0.080±0.081	0.076±0.042	0.056±0.028	0.072±0.031	0.082±0.042	0.085±0.042	0.068±0.044
Serum-IgG (g/l)	9.3± 2.3	10.2± 2.5	10.7±2.1[b]	8.8±1.7	10.5±3.9	9.9±2.4	11.7± 2.1	10.3± 1.8
CSF/serum albumin	5.3± 1.7[b]	3.9± 0.9	5.6±2.4	4.2±1.2	4.5±2.2	4.2±1.0	4.8± 1.4	4.4± 1.5
IgG-index	1.8± 0.6	1.9± 1.6	1.3±0.7	1.6±0.9	1.8±0.9	2.0±1.1	1.7± 1.0	1.3± 0.5
IgG_{SYN} (mg/d)	29 ± 20	27 ± 39	20 ± 18	17 ± 14	22 ± 11	28 ± 20	26 ± 21	20 ± 19
log_2 CSF ABA titre	-1.2± 1.6 (n = 9)	-1.9± 1.4 (n = 10)	-1.9±1.3 (n = 14)	-2.2±1.1 (n = 13)	-1.4±1.9 (n = 7)	-1.9±1.4 (n = 7)	-1.0± 0.6[c] (n = 6)	-2.5± 1.1 (n = 8)
log_2 serum ABA titre	2.1± 2.0 (n = 8)	1.5± 2.0 (n = 6)	1.5±1.8 (n = 13)	0.5±0.9 (n = 11)	1.1±1.8 (n = 7)	0.9±1.2 (n = 7)	1.4± 2.0 (n = 8)	1.4± 1.8 (n = 7)

a Cells x 10^6/l
b Higher than non-disabled controls, $P < 0.05$ (Student's t test, unpaired)
c Higher than non-disabled controls, $P < 0.05$ (Mann-Whitney U test)

barrier, as evidenced by the CSF/serum albumin ratios (range, 0.0021 to 0.0113). In these cases means of IgG-index and IgG_{SYN} values are relatively concordant.

Antibrain antibodies (ABA)

All paired CSF and serum samples discussed above and 55 additional serum samples from patients with neurological diseases other than MS (Table 1) were tested in the complement fixation test employing two different antigen preparations: a homogenate of white and gray matter of human brain and a cholesterol-enriched lipid extract of human brain (TLA). These heterogeneous preparations were chosen in order to detect the widest possible range of ABA specificities.

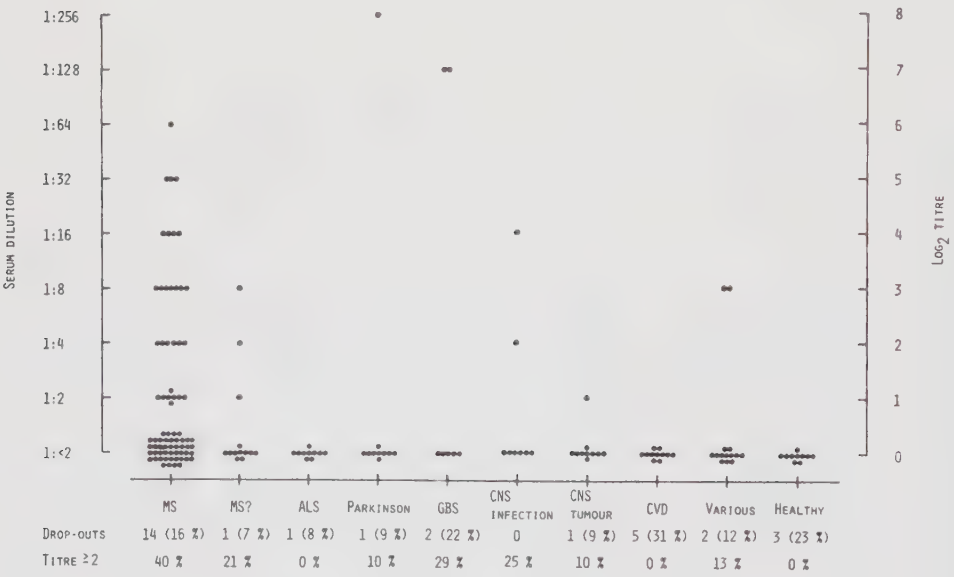

Figure 1. Results of ABA titrations against brain homogenate of serum from various patient groups and healthy controls. MS=clinically definitive MS, MS?=probable MS, ALS=amyotrophic lateral sclerosis, GBS=Guillain-Barré syndrome, CVD=cerebrovascular disease.

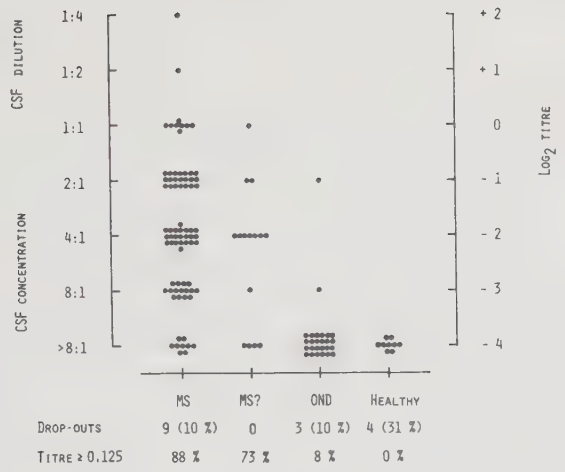

Figure 2. Results of ABA titrations against brain homogenate of CSF from various patient groups. Abbreviations as in Fig. 1. OND = other neurological diseases.

Figures 1 and 2 display the antibody titres against the brain homogenate in serum and CSF from all patient groups. Mean $\log_2$ titres $\pm$ SD in the various groups are given in the tables. In the computations of mean titres, CSF titres below 0.125 were assigned a titre of 0.0625 ($\log_2$ titre = -4), the next concentration step. Similarly serum titres <2 were assigned a titre of 1 ($\log_2$ titre = 0). The proportion of CSF titres $\geq$ 0.125 (i.e. having detectable ABA) was significantly higher in patients with MS than in patients with other neurological diseases and in healthy controls ($P < 0.0001$). A statistically significant but less pronounced difference in the proportion of serum titres above baseline was also found. The importance of working with concentrated CSF is evident from Fig. 2, and it is conceivable that the range of serum titres in MS is severely restricted by the limit of detection.

Serum and CSF samples reacting with TLA were rare in the MS groups (serum 3 %; CSF 13 % in the combined MS groups). Many of

them also reacted with the brain homogenate and this topic, therefore, was not further studied here.

Anticomplementary activity precluded the evaluation of ABA titres in 14 (16 %) serum samples and 9 (10 %) CSF samples from the group of clinically definitive MS. Among the probable MS cases, one serum sample was anticomplementary. The proportion of drop-outs because of anticomplementary activity among the other groups was similar; these are specified in Figures 1 and 2. The drop-outs in the group of clinically definitive MS did not differ conspiciously from the whole group concerning the variables that could be studied.

The reactions of positive samples in the control groups are presented in Table 10. High ABA titres were found in serum from two of nine patients in various phases of the Guillain-Barré syndrome. Both patients were in the acute phase of the disease. A further sample from one of the patients obtained 3 weeks after the first, when he was under corticosteroid treatment and much improved, showed a significantly reduced titre.

The specificity of the antibodies reacting with cerebral antigens in this study is largely unknown. However, none of the MS or control sera reacting with brain homogenate gave a positive reaction with a 1:100 (w/v) homogenate in saline of human kidney. Most serum samples reacting with TLA were tested against a standard Wasserman cardiolipin antigen. The two syphilitic patients had positive reactions and the patient with cerebral infarction and sequelae after poliomyelitis gave a weak reaction against this antigen (Table 10). All positive non-MS samples were tested against a suspension of CNS myelin, the structure giving the highest proportion of positive reactions with MS samples (Ryberg 1978). The only samples to give positive reactions were serum from the patient with epilepsy and the two with the Guillain-Barré syndrome (Table 10).

There was a tendency towards higher CSF ABA titres in younger patients and in those with an earlier onset of MS (Tables 5 and 6). Moreover, weakly positive correlations with mononuclear cell counts and IgG concentration in CSF existed. Only a slight difference in mean ABA titres between the two sexes were found despite the marked difference in mononuclear cell counts and IgG parameters in CSF (Tables 4 and 7).

Table 10. Positive reactions in the control patients

Diagnosis	Brain homogenate		TLA		Myelin		Cardiolipin	
	Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF
Cerebral infarction + sequelae after poliomyelitis	1:<2	2:1	1:4	>8:1	1:<1	n.d.	1:4	n.d.
B_{12} myelopathy	1:8	>8:1	1:<2	>8:1	1:<1	n.d.	n.d.	n.d.
Epilepsy	1:8	>8:1	1:64	>8:1	1:8	n.d.	1:<1	n.d.
Neurosyphilis	1:4	8:1	1:4	8:1	1:<1	n.d.	1:8	n.d.
Neurosyphilis	1:16	ac	1:<2	ac	1:<1	n.d.	1:128	n.d.
Parkinson´s disease	1:256	n.d.	1:<2	n.d.	1:<1	n.d.	n.d.	n.d.
Glioma	1:2	n.d.	n.d.	n.d.	1:<1	n.d.	n.d.	n.d.
Guillain-Barré	1:128	n.d.	1:<2	n.d.	1:>128	n.d.	n.d.	n.d.
Guillain-Barré	1:128	n.d.	1:128	n.d.	1:>128	n.d.	n.d.	n.d.

ac = anticomplementary

n.d. = not determined

Relatively high serum and CSF titres were found in patients with malignant courses ("2" and "3"). In the comparison of matched patients, this tendency was limited to those with a steady progression ("3") (Table 8). In these patients, GMT of serum ABA was 97 % higher ($P > 0.05$) and GMT of CSF ABA was 150 % higher ($P < 0.05$) than in matched patients with courses of type "1" and "2". These differences are much greater than for any other serum or CSF variable studied. GMT of serum ABA was about 30 % lower in exacerbating patients than in those in remission ($P > 0.05$). This difference was found in both sexes and persisted in the matched comparison. CSF ABA was virtually not correlated with clinical activity (Table 4 and 8).

There was a tendency for the disabled patients to have higher titres of serum ABA and CSF ABA than matched (and unmatched) non-disabled patients (Table 9). GMT of CSF ABA in patients reaching disability during the period 15-20 years after onset was 180 % higher than in matched MS patients still not disabled after 20 years. This difference is statistically significant ($p < 0.05$, Mann-Whitney U-test) and much greater than the difference in the other variables.

The group of probable MS contained seven patients with a duration of disease shorter than one year. These patients had CSF ABA titres similar to those with definitive MS and a longer duration of disease (Table 3). The serum titres, on the other hand, were generally low in these early cases, only one being over the baseline (one serum was anticomplementary). The situation was not different in three of these patients where samples were obtained within the first six weeks of clinically overt disease. The most striking feature in the early cases of probable MS was a marked mononuclear pleocytosis in most CSF samples (mean 27.4 cells x 10^6/l; SD 18.5).

CSF/serum ratio of ABA

All MS patients in whom both serum and CSF titres against brain homogenate were known are displayed in Fig. 3. The CSF/serum ABA ratio can be read by aid of the diagonal lines in all patients where both titres were above baseline. There was a weak positive correlation between serum ABA and CSF ABA (Table 6) which might have been more pronounced if the lower range of serum

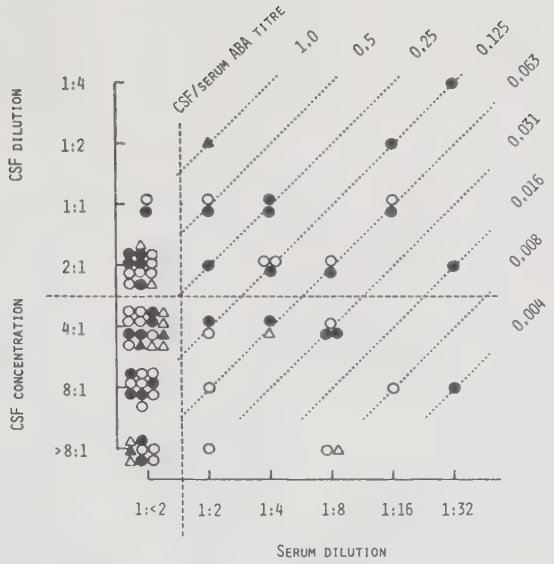

Figure 3. The relation between ABA titres in serum and CSF in MS patients. The dotted diagonal lines give the CSF/serum ABA ratios for paired serum and CSF titres. ○ = clinically definitive MS, not disabled. ● = clinically definitive MS, disabled. △ = probable MS, not disabled. ▲ = probable MS, disabled. The interrupted lines subdivide the patients into four groups: (a) those with a low serum titre and a "high" CSF titre, (b) those with "high" serum and CSF titres, (c) those with low serum and CSF titres, and (d) those with a "high" serum titre and a low CSF titre.

titres had not been cut by the limit of detection. None of the CSF/serum albumin ratios were much elevated (highest value 0.0113) (Table 4), indicating a very limited access of serum antibody to CSF. The wide range of CSF/serum ABA ratios therefore probably reflects varying proportions of intrathecal and extrathecal antibody synthesis (Ryberg 1980).

Subdivision of the clinically definitive MS patients according to Fig. 3 into four groups revealed an interesting pattern (Table 11). Patients in group a were predominantly male and some had a marked mononuclear pleocytosis in the CSF, high CSF-IgG, high IgG-index, and high IgG_{SYN}, giving elevated mean values of these variables. Also in group b many patients had high CSF

Table 11. Characterization of patients with clinically definitive MS subgrouped according to ABA titres in serum and CSF (Fig. 3)

Results are given as number and percentage or as means ± SD

	a n = 13	b n = 15	c n = 25	d n = 11
Male	8[a] (62 %)	5 (33 %)	7 (28 %)	3 (27 %)
Female	5 (38 %)	10 (67 %)	18 (72 %)	8 (73 %)
Clinical course 1	5 (38 %)	1[b] (7 %)	10 (40 %)	3 (27 %)
Clinical course 2	5 (38 %)	6 (40 %)	7 (28 %)	5 (45 %)
Clinical course 3	3 (23 %)	8 (53 %)	8 (32 %)	3 (27 %)
Disability +	6 (46 %)	10 (67 %)	10 (40 %)	5 (45 %)
Disability -	7 (54 %)	5 (33 %)	15 (60 %)	6 (55 %)
Clinical activity A	7 (54 %)	5 (33 %)	12 (48 %)	6 (55 %)
Clinical activity B	3 (23 %)	2 (13 %)	5 (20 %)	2 (18 %)
Age (yr)	39.5 ± 9.3	39.7 ± 7.5	42.6 ± 9.1	44.7 ± 14.3
Onset (yr)	26.1 ± 7.8	27.7 ± 7.8	30.7 ± 9.1	27.8 ± 8.8
Duration (yr)	13.4 ± 9.3	12.3 ± 7.2	11.8 ± 6.8	16.7 ± 8.4
CSF mononuclears[e]	13.0 ± 22.1	9.2 ± 15.1	6.2 ± 5.1[c]	2.7 ± 1.5
CSF-IgG (g/l)	0.094±0.064	0.088±0.039[c]	0.061±0.030	0.052±0.027
Serum-IgG (g/l)	11.0 ± 1.9	10.1 ± 2.0	9.7 ± 2.0	9.4 ± 3.9
CSF/serum albumin	5.2 ± 2.2	5.5 ± 1.8[d]	4.3 ± 1.6	4.5 ± 1.8
IgG-index	1.8 ± 1.2	1.6 ± 0.6	1.6 ± 0.7	1.2 ± 0.7
IgG_{SYN} (mg/d)	30 ± 30	28 ± 18	18 ± 13	12 ± 11

a Proportion of males higher than in c and in b + c + d, $P < 0.05$ (Fisher's exact test)

b Proportion of clinical course 1 lower than in a + c + d, $P < 0.05$ (Fisher's exact test)

c Higher than in d, $P < 0.05$ (Student's t test)

d Higher than in c, $P < 0.05$ (Student's t test)

e Cells x 10^6/l

mononuclear cell counts and high CSF-IgG levels. Moreover, relatively high CSF/serum albumin ratios were found in this group indicating some impairment of the blood-brain barrier. This agrees with the high proportion of disabled patients (67 %) and malignant courses ("2" and "3" comprising 93 %) in this group with high serum and CSF ABA titres. Patients in group c, which had the lowest proportion of disabled (40 %), had somewhat lower CSF mononuclear cell counts and IgG parameters in CSF than the whole MS group. This tendency was much more marked in group d where patients were generally somewhat older and had a longer mean duration of disease than in groups a, b, and c.

Patients without oligoclonal bands in CSF

For practical reasons, it was not possible to perform electrophoresis on the same CSF samples that were used for the ABA determination. However, the result of agar gel electrophoresis on other CSF samples, routinely performed in this laboratory as described by Link (1973), was known for 85 patients with clinically definitive MS. Six of them (7 %) had less than two bands in the γ-region on electrophoresis of one or more CSF samples. These patients had very low CSF mononuclear cell counts (mean, 2.8×10^6/l; SD, 2.9), CSF-IgG (mean, 0.035 g/l; SD, 0.016), CSF/serum albumin (mean, 3.8; SD, 1.3), IgG-index (mean 0.8; SD, 0.1), and IgG_{SYN} (mean, 3.4 mg/d; SD, 5.1). However, the GMT of ABA in serum and CSF were practically the same as in patients with oligoclonal bands, indicating the relative independence of these variables. Half of the patients without oligoclonal bands in CSF were disabled.

DISCUSSION

The principal purpose of the present study was to find a possible relation between ABA, on one hand, and clinical and laboratory variables in MS, on the other. Interest centred on patients with clinically definitive MS (McDonald and Halliday 1977), but a number of probable MS patients were included to shed light on the dynamics of ABA in the earliest phases of the disease. The representation of a variety of diseases with

definitive CNS pathology in the control group should provide an adequate basis for a discussion of ABA in terms of cause and effect.

Background data of the MS patients

With respect to sex, age, age at onset of MS, duration of disease and disability the 87 patients with clinically definitive MS represent patients seen at a neurological outpatient department rather than the entire MS population.

The finding of more pronounced deviations in the routine CSF variables in younger MS patients, in those with an early onset, and in those with a shorter duration of disease largely agrees with earlier reports (Link and Müller 1971; Olsson et al. 1976), although the interrelation between the time variables in the present material complicated the evaluation of such relations.

Differences in the laboratory variables between patients with and those without disability, with different courses of the disease, and in exacerbation and remission were to some extent dependent on differences in the time variables. However, in patients matched for the temporal factors, a higher mean CSF/serum albumin ratio, a measure of the condition of the blood-brain barrier (Tibbling et al. 1977), was found in those with greater disability and more malignant courses of the disease. This elevation could in many cases explain differences in mean CSF-IgG concentration ($r = 0.44$, $p < 0.001$), which, however, was also closely correlated with the two indicators of intrathecal IgG synthesis: IgG-index ($r = 0.72$, $p < 0.001$) and IgG_{SYN} ($r = 0.98$, $p < 0.001$). The positive correlation between the latter variable and CSF lymphocytes reported by Tourtellotte (1975) agrees with the present results, but the claimed positive correlation with age and duration of disease did not apply to this material (Table 6).

The higher means of CSF mononuclear cell count, CSF-IgG, CSF/serum albumin ratio, and IgG_{SYN} in male MS patients could not be explained by differences in age, age at onset of MS, duration of disease, and a number of clinical variables (Table 7). This topic has not received much attention in other investigations. As an exception, Schwarz et al. (1970) found that male MS patients

had higher total protein levels in CSF than had female patients. However, male patients had lower relative IgG concentrations in CSF.

ABA

The complement fixation assay employed in this investigation has proved reliable in earlier work in this field. Although normal human IgG is known to bind in a nonspecific way to some CNS components (Aarli et al. 1975), several observations indicate that this is not the basis for the findings with the complement fixation method. (a) Positive reactions are found only in some sera, mostly from MS patients, and most sera give no reaction even undiluted. (b) No correlation was found between IgG level and ABA titre in MS sera in the present study. (c) There was a very dissimilar frequency of positive reactions among MS CSF and non-MS CSF even in tests with a standardized IgG concentration (Ryberg 1976, 1978). (d) There was a virtual lack of correlation between CSF/serum ratios for IgG and ABA (Ryberg 1980). (e) Different MS samples show distinct reaction patterns, some being definitively shown to depend on reactions with separate antigens (Ryberg 1978). In combination with the demonstration of the IgG nature of the CSF and serum reactant (Ryberg and Kronvall 1981), this latter finding provides firm support for the antigen-antibody nature of the reaction.

In order to pick up the largest possible number of ABA specificities, two heterogenous antigen preparations from human brain were used. The sensivity of the assay was sufficient to demonstrate a positive CSF reaction with the brain homogenate in most MS patients when concentrated material was used, but was probably not enough to cover the lower range of serum reactions (Tables 1 and 2). The test could be a useful diagnostic aid, as positive reactions in non-MS patients were rare and apparently limited to certain conditions. The results should help resolve some of the prevailing uncertainty regarding the occurrence of antibodies to brain constituents in MS, as discussed by Lumsden (1972) and by Aarli and Tönder (1980).

The finding of high ABA titres and reactivity with CNS myelin in two of nine sera from patients with the Guillain-Barré syndrome, a usually monophasic demyelinating disease of both the peripheral

and central nervous system, is of great interest and confirms the reports of Melnick (1963) and Nyland and Aarli (1978). The discordant reaction with brain lipids in the two patients seems to implicate a heterogeneous autoimmune response in this disease, similar to the situation in MS (Ryberg 1978).

No extensive analysis of the specificity of antibodies reacting with the two crude brain antigen preparations was carried out in this study, and the specificities in non-MS patients could differ from those in MS patients (Table 10).

The proportion of MS samples reactive with TLA in this and an earlier study (Ryberg 1978) was clearly lower than the proportion of samples reacting with the brain homogenate. Some of the samples gave a reaction of similar titre with both antigen preparations probably because the lipid antigen is exposed on some cerebral membrane. The TLA preparation was formulated to fit the requirements of antibodies to sulfatide in one MS serum, and it is not unlikely that this composition was suboptimal for the detection of antibodies to other haptens.

Similar to several other laboratory variables, there was a tendency for higher ABA titres in CSF in younger patients and in those with an earlier onset of MS (Tables 5 and 6). Titres in CSF obtained shortly after the clinical onset of the disease (which is not necessarily the earliest manifestation of MS) did not significantly differ from those in samples obtained later in its course.

CSF ABA titres were somewhat correlated with CSF-IgG levels and serum ABA titres (Table 6), but for reasons mentioned above, it is unlikely in many cases that a significant portion of CSF ABA derived from blood.

Taken together, patients with clinically definitive and probable MS showed a tendency towards increasing ABA titres in serum but not in CSF. This agrees with the findings in the adjoining longitudinal study (Ryberg 1981) and might reflect a progressive dissemination of the autoimmune process beyond the CNS.

In agreement with the longitudinal study, no significant difference in ABA titres were found between patients in exacerbation and in those in remission. Actually serum GMT was somewhat lower in patients in exacerbation, and neither group has as high

GMT as patients in chronic progression. This is at variance with the results of Lisak et al. (1975) and of Panitsch et al. (1980). The former authors reported higher levels of serum antibodies to CNS myelin in MS patients in exacerbation. The results, however, were obtained by an immunofluorescent technique, which is now known to be liable to false positive reactions (Traugott et al. 1979; Kennedy and Lisak 1981) because of an unspecific affinity of normal human IgG for CNS constituents (Aarli et al. 1975). Panitsch et al. (1980) found elevated levels of IgG binding to insolubilized myelin basic protein in CSF from MS patients, particularly in those in exacerbation, but even higher levels in subacute sclerosing panencephalitis. The risk of false positive reactions in this assay by IgG aggregates or immune complexes has been pointed out by Sindic et al. (1980).

Patients with a chronic progressive course had higher GMT of ABA in CSF than had matched control patients with remittent courses. Serum and CSF ABA titres were usually higher in disabled patients than in patients not disabled after a similar duration of MS, implying an association with more malignant courses. This interesting finding can be explained as a phenomenon secondary to the disease process. Thus, several mechanisms, largely attributable to an excessive immunogenic stimulation and/or a defective immunoregulation, can give rise to autoantibodies in experimental models and human disease (Allison 1977). However, it seems that the mere destruction of CNS tissue is usually not sufficient to induce a humoral autoimmune response as seen from the results in the control patients.

The association between ABA and a malignant course might be of a more important nature, as, irrespective of the mechanism eliciting ABA in MS, these auto-antibodies are potentially pathogenic in one or more of the following ways:

(1) Direct action of antibody. Support for this mechanism is only indirect and derived from experiments with decomplemented serum from animals with experimental allergic encephalomyelitis (EAE). Such serum induces myelin swelling and aberrant myelination in organotypic cultures of mammalian CNS (Raine et al. 1978).

(2) Interaction with complement. In contrast to the complement-dependent in vitro demyelinating and gliotoxic activities in EAE serum (Appel and Bornstein 1964; Berg and Bergstrand 1968),

the corresponding activity in MS serum was found only exceptionally to be immunoglobulin mediated (Grundke-Iqbal and Bornstein 1980). However, IgG from several MS CSF caused demyelination in living tadpoles (Tabira et al. 1977) and the concept of an IgG mediated activation of complement in the pathogenesis of MS is supported by the observation of IgG and complement firmly bound within MS plaques (Woyciechowska and Brzosko 1977).

Probably unrelated to the demyelinating process, MS sera exhibited complement-dependent, reversible neuroelectric blocking activity in mammalian CNS explants (Bornstein and Crain 1965). In an amphibian in vitro model more specific for demyelinating disease (Cerf and Carels 1966), the activity seemed to be IgG mediated (Schauf and Davis 1978).

Antibodies to gangliosides, which are frequently found in MS sera (Mullin et al. 1980) are known to induce a number of biological effects when injected in the brain of experimental animals, possibly interacting with receptor functions of G_{M1} ganglioside (Rapport et al. 1980). Similarly, antibodies to sulfatide, which were demonstrated in two of 60 MS patients (Ryberg 1978), have now been found to block opiate mediated effects in rats (Craves et al. 1980). However, it is not known whether or not these in vivo effects are amplified by complement.

(3) Antibody-dependent cell-mediated cytotoxicity (ADCC). This was thought to be the mechanism in experiments where normal human lymphocytes in the presence of MS serum, or MS IgG attacked chicken erythrocytes coated with myelin basic protein (Frick and Stickl 1980), rat brain cultures (Morell 1980), or a rat glioma cell line (Mar 1980). The paucity or virtual absence of lymphocytes in the active edge of the MS plaque (Prineas and Connell 1978) argues against the importance of this mechanism at this site.

(4) Interaction with phagocytic cells. Phagocytic attack on myelin by cells identified as astroglia and microglia has recently been observed in MS plaques (Prineas and Raine 1976, Prineas and Connell 1978). It was suggested (Raine 1977) that macrophages in MS brain attack myelin and oligodendroglia opsonized by specific immunoglobulins, which, however, were not documented. The demonstration of ABA in MS and the finding in MS plaques of

macrophages with Fc receptor activity (Nyland et al. 1980) and intracellular, probably ingested, IgG (Esiri 1980) are important steps in the validation of this model. Further support is provided by the rabbit eye model, where stripping of myelin by a phagocytic process was apparently dependent on anti-CNS antibodies (Brosnan et al. 1977). Conceivably, IgG non-specifically bound to brain membranes by the Fc part (Aarli et al. 1975) would give a less efficient opsonization than ABA specifically bound. The active role of phagocytosis in demyelinating diseases, however, is not unambiguous and macrophages might merely be scavengers of damaged tissue. Such a role is well known in other conditions and was found probable when primary demyelination was induced by injection into the guinea-pig spinal cord of antiserum to myelin (Williams et al. 1980). Antiserum-mediated demyelination preceded phagocytosis in this model.

Macrophages stimulated by T-cell products or involved in antibody mediated phagocytosis exert a number of extracellular effects (Ögmundsdottir and Weir 1980). Proteolytic and other enzymes which are increased in and around MS plaques and in MS CSF (Davison and Cuzner 1977) are thought in part to derive from stimulated phagocytic cells. Such enzymes can digest myelin basic protein in isolated myelin (Cammer et al. 1978) and might play a role in vivo as well (Whitaker 1978); some of them are perhaps related to the non-immunoglobulin in vitro demyelinating factors (Grundke-Iqbal and Bornstein 1980). Thus, ABA might play a role in demyelination also by mechanisms of this kind.

A protective role for ABA?

Despite the many pathogenic possibilities for ABA, they have rather been associated with a relative resistance to EAE (Lisak et al. 1970 ; Lebar and Voisin 1974). The protection against EAE can be transferred by injection of sera containing complement fixing ABA in animals challenged with encephalitogenic preparations (Paterson et al. 1965; Salvin and Liauw 1968), but the protective agents and mechanisms are not known. Anyhow, it is possible that both synergistic and antagonistic effects are exerted in the target tissue by ABA of identical or different properties.

CONCLUSIONS

Largely confirming earlier reports, deviations in routine CSF variables in this MS material were on average more pronounced in younger patients, in those with an earlier onset and/or a shorter duration of disease. Moreover, patients with a steady progress had on average a less efficient blood-brain barrier for proteins than had patients in a remittent course. This explains most of the differing CSF-IgG levels in these groups. Male MS patients had often more marked alterations in their CSF than had female patients.

The presence of complement fixing ABA in serum and CSF is a distinctive feature of MS and might have diagnostic applications.

ABA titres appear to be correlated with a more malignant course and may be a result of the disease and/or have pathogenic effects.

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REFERENCES

Aarli, J.A., S.R. Aparicio, C.E. Lumsden and O. Tönder (1975): Binding of normal human IgG to myelin sheaths, glia and neurons. Immunology, 28:171-185.

Aarli, J.A. and O. Tönder (1980): Immunological aspects of neurological diseases. S. Karger, Basel.

Acheson, E.D. (1972): The epidemiology of multiple sclerosis: In: D. McAlpine, C.E. Lumsden, and E.D. Acheson (Eds.), Multiple sclerosis - A Reappraisal, 2nd edition, Churchill Livingstone, Edinburgh & London, pp. 3-80.

Allison, A.C. (1977): Autoimmune diseases: Concepts of pathogenesis and control: In: N. Talal (Ed.), Autoimmunity. Genetic, immunologic, virologic and clinical aspects. Academic Press, New York, pp. 91-139.

Appel, S.H. and M.B. Bornstein (1964): The application of tissue culture to the study of experimental allergic encephalomyelitis. II. Serum factors responsible for demyelination. J. exp. Med. 119:303-312.

Arnason, B.G.W. and J. Antel (1978): Suppressor cell function in multiple sclerosis. Ann. Immunol. (Inst. Pasteur), 129 C: 159-170.

Berg, O. and H. Bergstrand (1968): Different types of antibodies with a gliotoxic effect in serum from animals with experimental allergic encephalomyelitis. Acta path. microbiol. scandinav., 73:195-210.

Bornstein, M.B. and S.M. Crain (1965): Functional studies of cultured brain tissues as related to "demyelinative disorders". Science, 148:1242-1244.

Brosnan, C.F., G.L. Stoner, B.R. Bloom and H.M. Wisniewski (1977): Studies on demyelination by activated lymphocytes in the rabbit eye. II. Antibody-dependent cell-mediated demyelination. J. Immunol. 118:2103-2110.

Cammer, W., B.R. Bloom, W.T. Norton and S. Gordon (1978): Degradation of basic protein in myelin by neutral proteases secreted by stimulated macrophages: A possible mechanism of inflammatory demyelination. Proc. Natl. Acad. Sci. (Wash.) 75:1554-1558.

Cerf, J.A. and G. Carels (1966): Multiple sclerosis: Serum factor producing reversible alterations in bioelectric responses. Science 152:1066-1068.

Craves, F.B., B. Zalc, L. Leybin, N. Baumann and H. Loh (1980): Antibodies to cerebroside sulfate inhibit the effects of morphine and β-endorphin. Science 207:75-76.

Cremer, N.E., K.P. Johnson, G. Fein and W.H. Likosky (1980): Comprehensive viral immunology of multiple sclerosis. II. Analysis of serum and CSF antibodies by standard serologic methods. Arch. Neurol. (Chic.) 37:610-615.

Davison, A.N. and M.L. Cuzner (1977): Immunochemistry and biochemistry of myelin. Brit. Med. Bull. 33:60-66.

Delpech, B. and E. Lichtblau (1972): Étude quantitative des immunoglobulines G et de l'albumine du liquide céphalorachidien. Clin. chim. Acta 37:15-23.

Esiri, M.M. (1977): Immunoglobulin-containing cells in multiple-sclerosis plaques. Lancet 2: 478-480.

Esiri, M. (1980): Multiple sclerosis: A quantitative and qualitative study of immunoglobulin-containing cells in the central nervous system. Neuropath. Appl. Neurobiol. 6:9-21.

Folch-Pi, J. (1972): Proteolipids. In: A.N. Davison, P. Mandel and J.G. Morgan (Eds.). Functional and Structural Proteins of the Nervous System. Plenum Press. New York, NY, pp. 171-189.

Frick, E. and L. Scheid-Seydel (1958): Untersuchungen mit J^{131}-markiertem γ-Globulin zur Frage der Abstammung der Liquoreiweisskörper. Klin. Wschr. 36:857-863.

Frick, E. and H. Stickl (1980): Antibody-dependent lymphocyte cytotoxity against basic protein of myelin in multiple sclerosis. J. neurol. Sci. 46:187-197.

Fulton, F. and K.R. Dumbell (1949): The serological comparison of strains of influenza virus. J. gen. Microbiol. 3:97-111.

Grundke-Iqbal, I. and M.B. Bornstein (1980): Multiple sclerosis: Serum gamma globulin and demyelination in organ culture. Neurology (Minneap.) 30:749-754.

Hallpike, J.F. (1972): Enzyme and protein changes in myelin breakdown and multiple sclerosis. Progr. Histochem. Cytochem. 3:179-218.

Källén, B., B. Löw and O. Nilsson (1975): Mixed leukocyte reaction and HL-A specificity at multiple sclerosis. Acta neurol. scand. 51:184-192.

Kennedy, P.G.E. and R.P. Lisak (1981): Do patients with demyelinating diseases have antibodies against human glial cells in their sera? J. Neurol. Neurosurg. Psych. 44:164-167.

Lebar, R. and G.A. Voisin (1974): Studies on auto-immune encephalomyelitis in the guinea pig. Int. Arch. Allergy 46:82-103.

Link, H. (1967): Immunoglobulin G and low molecular weight proteins in human cerebrospinal fluid. Acta neurol. scand. 43 (Suppl. 28):1-136.

Link, H. (1972): Oligoclonal immunoglobulin G in multiple sclerosis brains. J. neurol. Sci. 16:103-114.

Link, H. (1973): Comparison of electrophoresis on agar gel and agarose gel in the evaluation of gamma-globulin abnormalities in cerebrospinal fluid and serum in multiple sclerosis. Clin. chim. Acta 46:383-389.

Link, H. and R. Müller (1971): Immunoglobulins in multiple sclerosis and infections of the nervous system. Arch. Neurol. (Chic.) 25:326-344.

Link, H. and G. Tibbling (1977): Principles of albumin and IgG-analysis in neurological disorders. III. Evaluation of IgG synthesis within the central nervous system in multiple sclerosis. Scand. J. Clin. Lab. Invest. 37:397-401.

Lisak, R.P. (1980): Multiple sclerosis: Evidence for immunopathogenesis. Neurology (Minneap.) 20 (2):99-105.

Lisak, R.P., G.A. Falk, R.G. Heinze, M.W. Kies and E.C. Alvord (1970): Dissociation of antibody production from disease suppression in the inhibition of allergic encephalomyelitis by myelin basic protein. J. Immunol. 104:1435-1446.

Lisak, R.P., B. Zwiman and M. Norman (1975): Antimyelin antibodies in neurologic diseases. Immunofluorescent demonstration. Arch. Neurol. (Chic.) 32:163-167.

Lowenthal, A. (1964): Agar gel electrophoresis in neurology. Elsevier Publishing Company, Amsterdam.

Lumsden, C.E. (1972): The clinical immunology of multiple sclerosis. In: D. McAlpine, C.E. Lumsden and E.D. Acheson (Eds.), Multiple Sclerosis - A Reappraisal, 2nd edition, Churchill Livingstone, Edinburgh, London, pp. 512-621.

Mancini, G., A.O. Carbonara and J.F. Heremans (1965): Immunochemical quantification of antigens by single radial immunodiffusion. Immunochemistry 2:235-254.

Mar, P. (1980): Antibody-dependent cellular cytotoxicity in multiple sclerosis. J. neurol. Sci. 47:285-303.

Mattson, D.H., R.P. Roos and B.G.W. Arnason (1980): Isoelectric focusing of IgG eluted from multiple sclerosis and subacute sclerosing panencephalitis brains. Nature (Lond.)287:335-337.

McDonald, W.J. and A.M. Halliday (1977): Diagnosis and classification of multiple sclerosis. Brit. Med. Bull. 33:4-8.

Melnick, S.C. (1963): Thirty-eight cases of the Guillain-Barré syndrome: An immunological study. Br.med. J. 1:368-373.

Mies, H.-J. (1953):Einegung von Liquor cerebrospinalis als Vorbereitung zur Papierelectro-Phorese. Klin. Wschr. 31:159-161.

Morell, R.M. (1980): Immunoglobulin (Ig) from multiple sclerosis (MS) lymphocyte surface confers cytotoxic potential on normal mononuclear cells. Neurology (Minneap.), 30:398.

Müller, R. (1949): Studies on multiple sclerosis. Acta med.scand. 133 (Suppl. 222):1-214.

Mullin, B.R., A.J. Montanaro, J.D. Reid and N. Nishimura (1980): Interaction of multiple sclerosis serum with liposomes containing ganglioside G_{M1}. Ann Neurol. 7:587-590.

Nordal, H.J. and B. Vandvik (1977): Evidence of local synthesis of smooth-muscle antibodies in the central nervous system in isolated cases of multiple sclerosis and chronic lymphocytic meningo-encephalitis. Scand. J. Immunol. 6:327-334.

Nordal, H.J., B. Vandvik and E. Norrby (1978): Demonstration of electrophoretically restricted virus-specific antibodies in serum and cerebrospinal fluid by imprint electroimmunofixation. Scand. J. Immunol. 7:381-388.

Norrby, E., H. Link, J.-E. Olsson, M. Panelius, A. Salmi and B. Vandvik (1974): Comparison of antibodies against different viruses in cerebrospinal fluid and serum samples from patients with multiple sclerosis. Infect. Immun. 10:688-694.

Nyland, H. and J.A. Aarli (1978): Guillain-Barrē syndrome: demonstration of antibodies to peripheral nerve tissue. Acta neurol. scand. 58:35-43.

Nyland, H., R. Matre and S. Mørk (1980): Fc receptors on microglial lipophages in multiple sclerosis. N. Engl. J. Med. 302:120-121.

Ögmundsdottir, H.M. and D.M. Weir (1980): Mechanisms of macrophage activation. Clin. exp. Immunol. 40:223-234.

Olsson, J.-E., H. Link and R. Müller (1976): Immunoglobulin abnormalities in multiple sclerosis. Relation to clinical parameters: disability, duration and age of onset. J. neurol. Sci. 27:233-245.

Panitsch, H.S., C.J. Hooper and K.P. Johnson (1980): CSF antibody to myelin basic protein. Measurement in patients with multiple sclerosis and subacute sclerosing panencephalitis. Arch. Neurol. (Chic.) 37:206-209.

Paterson, P.Y., A.F. Jacobs and E.M. Coia (1965): Complement-fixing antibrain antibodies and allergic encephalomyelitis. II. Further studies concerning their protective role. Ann. N.Y. Acad. Sci. 124(I):292-298.

Prineas, J.W. (1979): Multiple sclerosis: Presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. Science 203:1123-1125.

Prineas, J.W. and F. Connell (1978): The fine structure of chronically active multiple sclerosis plaques. Neurology (Minneap.) 28(2):68-75.

Prineas, J.W. and C.S. Raine (1976): Electron microscopy and immunoperoxidase studies of early multiple sclerosis lesions. Neurology 26(2):29-32.

Raine, C.S. (1977): The etiology and pathogenesis of multiple sclerosis: Recent developments. Pathobiol. Ann. 7:347-384.

Raine, C.S., M. Diaz, M. Pakingan and M.B. Bornstein (1978): Antiserum-induced dissociation of myelinogenesis in vitro. An ultrastructural study. Lab. Invest. 38:397-403.

Rapport, M.M. and L. Graf (1967): Preparation and testing of lipids for immunological study. In: C.A. Williams and M.W. Chase (Eds.). Methods in Immunology and Immunochemistry, Vol 1. Academic Press, New York, N.Y. pp. 187-196.

Rapport, M.M., S.E. Karpiak and S.P. Mahadik (1980): Perturbation of CNS functions by antibodies to gangliosides. Speculations on biological roles of ganglioside receptors. In: L. Svennerholm, P. Mandel, H. Dreyfus and P.-F. Urban (Eds.), Symposium on structure and function of gangliosides, Plenum Press, New York & London, pp. 335-338.

Reinherz, E.L., H.L. Weiner, S.L. Hansen, J.A. Cohen, J.A.Distaso and S.F. Schlossman (1980): Loss of suppressor T cells in active multiple sclerosis. N. Engl. J. Med. 303:125-129.

Ross, C.A.C., J.A.R. Lenman and C. Rutter (1965): Infective agents and multiple sclerosis. Br. Med. J. 1:226-229.

Ryberg, B. (1976): Complement-fixing antibrain antibodies in multiple sclerosis. Acta neurol. scand. 54:1-12.

Ryberg, B. (1978): Multiple specificities of antibrain antibodies in multiple sclerosis and chronic myelopathy. J. neurol. Sci. 38:357-382.

Ryberg, B. (1980): Intrathecal and extrathecal production of antibrain antibodies in multiple sclerosis. J. neurol. Sci. 48:1-8.

Ryberg, B. (1981): A longitudinal study of antibrain antibodies in multiple sclerosis. Manuscript.

Ryberg, B. and G. Kronvall (1981): Immunoglobulin characterization by bacterial absorption of antibrain antibodies in multiple sclerosis. Eur. Neurol. In press.

Sandberg-Wollheim, M. (1974): Immunoglobulin synthesis in vitro by cerebrospinal fluid cells in patients with multiple sclerosis. Scand. J. Immunol. 3:717-730.

Salvin, S.B. and H.L. Liauw (1968): Immunologic unresponsiveness, immunity and enhancement in experimental allergic encephalomyelitis. J. Immunol. 101:33-42.

Schauf, C.L. and F.A. Davis (1978): The occurrence, specificity, and role of neuroelectric blocking factors in multiple sclerosis. Neurology (Minneap.) 28 (2):34-39.

Schwartz, S., H.P. Rieder and R. Wüthrich (1970): The protein fractions in cerebrospinal fluid in the various states of multiple sclerosis. Eur. Neurol. 4:267-282.

Sindic, C.J.M., C.L. Cambiaso, P.L. Masson and E.C. Laterre (1980): The binding of myelin basic protein to the Fc region of aggregated IgG and to immune complexes. Clin. Exp. Immunol. 41:1-7.

Sörnäs, R. (1967): A new method for cytological examination of cerebrospinal fluid. J. Neurol. Neurosurg. Psychiatry 30:568-577.

Symington, G.R., I.R. Mackay, S. Wittingham, J. White and J.D. Buckley (1978): A "profile" of immune responsiveness in multiple sclerosis (1978). Clin. exp. Immunol.31:141-149.

Tabira, T., F.J. Wolfgram, H. de F. Webster, S.H. Wray and D.E. McFarlin (1977): Myelinotoxicity of CSF fractions from multiple sclerosis patients tested in an in vivo model. Neurology (Minneap.) 27:374.

Tibbling, G., H. Link and S. Öhman (1977): Principles of albumin and IgG analysis in neurological disorders. I. Establishment of reference values. Scand. J. clin. Lab. Invest. 37:385-390.

Tourtellotte, W.W. (1975): What is multiple sclerosis? Laboratory criteria for diagnosis. In: A.N. Davison, J.H. Humphrey, A.L. Liversedge, W.J. McDonald and J.S. Porterfield (Eds.). Multiple Sclerosis Research, Her Majesty´s Stationary Office, London, pp. 9-26.

Traugott, V., D.S. Snyder and C.S. Raine (1979): Oligodendrocyte staining by multiple sclerosis serum is nonspecific. Ann. Neurol. 6:13-20.

Vandvik, B., E. Norrby and H.J. Nordal (1979): Optic neuritis: Local synthesis in the central nervous system of oligoclonal antibodies to measles, mumps, rubella and herpes simplex viruses. Acta neurol. scand. 60:204-213.

Whitaker, J.N. (1978): The distribution of myelin basic protein in central nervous system lesions of multiple sclerosis and acute experimental allergic encephalomyelitis. Ann. Neurol. 3:291-298.

Williams, R.M., S. Krakowka and A. Koestner (1980): In vivo demyelination by antimyelin antibodies. Acta Neuropathol (Berl.) 50:1-8.

Woyciechowska, J.L. and W.J. Brzosko (1977): Immunofluorescence study of brain plaques from two patients with multiple sclerosis. Neurology (Minneap.) 27:620-622.

Wright, R., J.A. Morton and K.B. Taylor (1965): Immunological studies in multiple sclerosis: Incidence of circulating antibodies to dietary proteins and auto-antigens. Brit. Med. J. 1:491-492.

Paper VI

A LONGITUDINAL STUDY OF ANTIBRAIN ANTIBODIES IN MULTIPLE SCLEROSIS

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SUMMARY

The presence of complement-fixing antibrain antibodies is a distinctive feature of multiple sclerosis (MS). In a longitudinal study of 35 MS patients antibrain antibody titres in serum were followed for up to five years; in 18 of them also CSF titres were determined. No consistent correlations between antibrain antibody titres and clinical events were found. Thus, MS relapses are not caused by a general increase in antibrain antibody titres, and conversely the relapses did not cause a boosting of antibrain antibodies. Significant variations in the local plaque environment are, however, not ruled out by the present results.

INTRODUCTION

Complement-fixing antibrain antibodies (ABA) are demonstrable in serum and CSF in a large proportion of patients with multiple sclerosis (MS) and show a high degree of specificity for demyelinating disease (Ryberg 1981). The antibodies are usually of IgG class (Ryberg and Kronvall 1981). They represent several specificities (Ryberg 1978) and are produced both intra- and extrathecally (Ryberg 1980). Moreover, evidence for their correlation with a more malignant course of MS was recently found (Ryberg 1981). In the present study, ABA were titrated in series of serum and CSF samples from MS patients to elucidate their relation to clinical events.

MATERIAL AND METHODS

CSF and serum samples were collected from 12 male and 23 female MS patients, diagnosed according to the criteria of Müller (1949). The age of the patients when included in the study was 16 to 56 years (mean 36.8 years; S D = 8.5) and the disease had lasted for one month to 29 years (mean 9.1 years; S D = 7.3) at this time. The time spans between the first and the last sample in the sequences are given in Table 1. The pooled observation time amounts to about 93 years. During this time or within one month before obtaining the first sample, these patients suffered 105 exacerbations of the disease, verified on clinical examination and/or found highly probable from anamnestic data. Thirteen patients were in a stage of chronic progression during the whole or part of the study; in eight of them, the chronic progression started during the observation time. Fourteen patients were given a total of 37 courses of ACTH (in all 980 units intramusculary in tapering doses for three weeks) during the study. The courses were with few exceptions given during a clinical

Table 1. The time passed between the first and last samples in the sequences

Months	No. of patients
0 - 6	2
7 - 12	3
13 - 18	2
19 - 24	3
25 - 30	4
31 - 36	7
37 - 42	7
43 - 48	5
49 - 54	1
55 - 60	1

exacerbation. Altogether 281 serum samples from 35 patients and 144 CSF samples from 10 patients, were tested. The distribution of patients according to number of samples is presented in Table 2.

Serum and CSF samples were treated and analysed essentially as described previously (Ryberg 1981). White matter and grey matter homogenates in saline (1:100, w/v) were employed as antigen preparations, as found appropriate in pilot experiments.

CSF was tested at 0.5, 1, 2, 4, 8, 16, and 32 times the original IgG concentration.

ABA titres that were less than four times higher than titre of anticomplementary activity were discarded.

Table 2. Distribution of patients according to number of samples tested

	No. of samples in sequence													
	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Serum	1	5	5	3	4	3	3	2	2	3	2			2
CSF	3	1		2	2	2	2	2	1	2				1
Paired serum + CSF	3	1		3	4	2		2	1	1			1	

RESULTS

Stable or slowly increasing serum and CSF ABA titres were seen in most patients during prolonged observation periods covering relapses, remissions, latent phases, and chronically progressive courses.

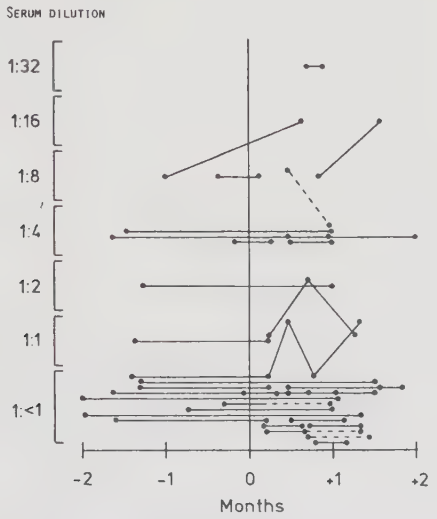

Fig. 1: Serum titre of ABA in exacerbating MS. All observations obtained in two or more samples within the indicated period before and/or after an attack are included. Excluded are single observations and titres obtained during a previous bout or within four weeks after its subsidence or within six weeks after the termination of an ACTH course. An interrupted line indicates that an ACTH course has been instituted.

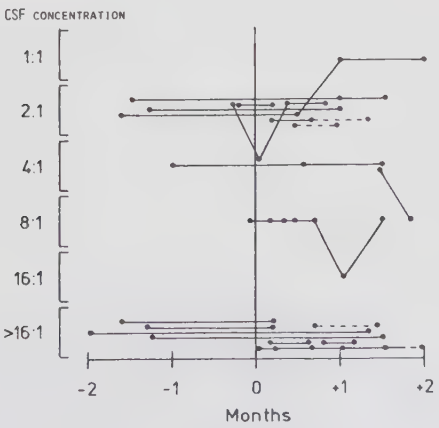

Fig. 2: CSF titre of ABA in exacerbating MS. For explanations, see Fig. 1.

Figures 1 and 2 show that no change in ABA greater than one twofold titre step occurred in serum or CSF in close relation to clinical exacerbations. Nor did the individual series reveal any tendency for a change in ABA titre preceding or following the relapses. One patient (Fig. 3) had a temporary rise and a subsequent fall in serum and CSF titres related to a period of frequent ACTH treated bouts. The ACTH courses were not likely to cause the increase in ABA titres, as this kind of treatment left the ABA titre unchanged in several other patients and was followed by a decrease in serum ABA titres in two patients, one of whom is presented in Fig. 4.

The relative stability of ABA titres during chronic progression is seen in Figures 5 and 6.

In 22 of the 35 serum sequences and in 7 of the 18 CSF sequences, the titre was persistently at or below the level of detection and did not allow a meaningful evaluation. Anticomplementary activity precluded the determination of ABA in a number of samples and many titres were obtained at times when they could not be related to a single, well-defined clinical event.

The geometric mean titre of sera with demonstrable ABA increased by about 20 per cent from the first to the third year of observation. This increase was not statistically significant, as found by Wilcoxon's test for pair differences. CSF titres did not show any similar trend.

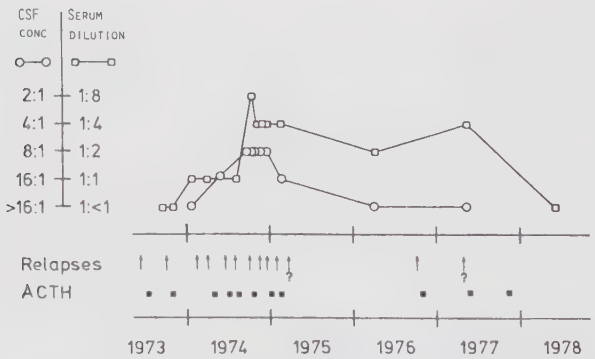

Fig. 3: Female patient born in 1947 with MS since 1966. During a period from 1973 to 1975 she had frequent relapses leaving her with a moderate disability. Most of the relapses were treated with ACTH. CSF and serum ABA showed a temporary rise during this time.

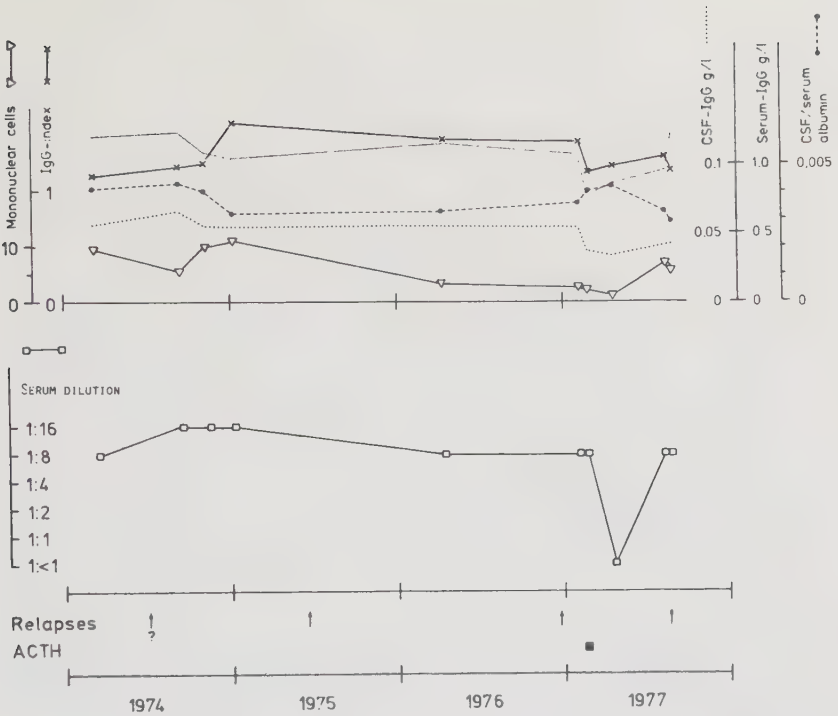

Fig. 4: Female patient born in 1939 with MS since 1968. In December 1976, she had a severe relapse leaving her much disabled until she had a remarkable improvement during ACTH treatment instituted 6 weeks after the relapse. CSF-IgG and serum-IgG fell about 30 % during ACTH treatment. ABA in serum showed a dramatic but delayed drop, being undetectable six weeks after the termination of ACTH treatment.

In the two patients reacting with a decreased ABA titre in serum in connection with ACTH treatment, there was an associated drop in the mononuclear cell count in CSF, CSF-IgG, serum-IgG, and IgG-index (Fig. 4). Except for these instances, there was virtually no parallelism in the individual sequences between ABA titres, on one hand, and mononuclear cell count, CSF-IgG, serum-IgG, CSF/serum albumin ratio, or IgG-index, on the other.

All samples in a sequence were assayed for ABA on the same day. Intra-experimental variation was evaluated by blind testing in triplicate of dilutions in veronal buffer of known positive samples. The variation in titre for each dilution was not more than two-fold.

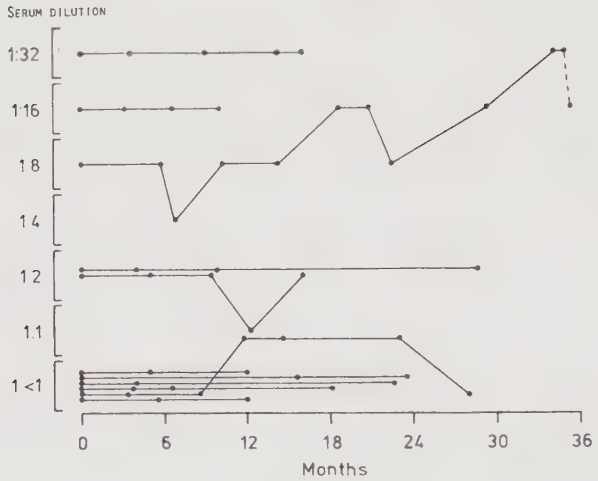

Fig. 5: Serum titre of ABA during chronic progression. The time scale gives the time passed after the first sample obtained during chronic progression.

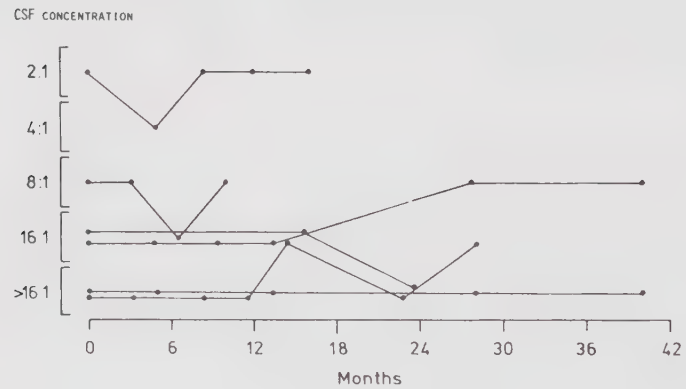

Fig. 6: CSF titre of ABA during chronic progression. For explanations, see Fig. 1 and Fig. 5.

DISCUSSION

The mechanisms behind acute relapses, remissions, and progressive courses in MS are not known. In view of the many ways by which ABA may influence the CNS (Ryberg 1981), it was deemed of interest to follow the ABA titres in serum and CSF in a longitudinal study. The fact that ABA titres were essentially constant before and during several exacerbations does not favour the idea of an active regulatory role for ABA in these clinical events. However, the MS lesions are anatomically limited, each plaque appearing to have its individual IgG composition (Esiri 1980; Mattson et al. 1980) and a change in ABA concentration, avidity, or specificity in the local environment of an active plaque is perhaps not reflected in serum or lumbar CSF. The findings do not rule out an effector role for ABA. A localized depression of some resistance factor (e.g., the blood-brain barrier) could permit an anatomically and temporally restricted action of a circulating antibody of constant titre.

The relative stability during and after relapses, which ABA share with various viral antibodies in serum (Sibley and Foley 1965; Reunanen et al. 1976) and antibodies involved in antibody-dependent cell-mediated cytotoxicity against target cells coated with myelin basic protein (Frick and Stickl 1980) suggests that the drop in suppressor cells in blood during MS exacerbations (Reinherz et al. 1980) is not sufficient to cause a general effect on humoral immunity. Because of the possible role of an excessive immunogenic stimulation by destruction of CNS tissue for the induction of ABA in MS, it is of interest to note that no separate bout caused a significant boosting of ABA.

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REFERENCES

Esiri, M. (1980): Multiple sclerosis: A quantitative and qualitative study of immunoglobulin-containing cells in the central nervous system. Neuropath. Appl. Neurobiol. 6:9-21.

Frick, E. and H. Stickl (1980): Antibody-dependent lymphocyte cytotoxicity against basic protein of myelin in multiple sclerosis. J. neurol. Sci. 46:187-197.

Mattson, D.H., R.P. Roos and B.G.W. Arnason (1980): Isoelectric focusing of IgG eluted from multiple sclerosis and subacute sclerosing panencephalitis brains. Nature (Lond.)287:335-337.

Müller, R. (1949): Studies on multiple sclerosis. Acta med. scand. 133 (Suppl. 222):1-214.

Reinherz, E.L., H.L. Weiner, S.L. Hansen, J.A. Cohen, J.A.Distaso, and S.F. Schlossmann (1980): Loss of suppressor T cells in active multiple sclerosis. N. Engl. J. Med. 303:125-129.

Reunanen, M., P. Arstila, H. Hakkarainen, J. Nikoskelainen, A. Salmi, and M. Panelius (1967): A longitudinal study on antibodies to measles and rubella viruses in patients with multiple sclerosis. A preliminary report. Acta neurol.scand. 54:366-370.

Ryberg, B. (1978): Multiple specificities of antibrain antibodies in multiple sclerosis and chronic myelopathy. J.neurol. Sci. 38:357-382.

Ryberg, B. (1980): Intrathecal and extrathecal production of antibrain antibodies in multiple sclerosis. J. neurol. Sci. 48:1-8.

Ryberg, B. (1981): Antibrain antibodies in multiple sclerosis. Relation to clinical variables. (To be sumbitted)

Ryberg, B. and G. Kronvall (1981): Immunoglobulin characterization by bacterial absorption of antibrain antibodies in multiple sclerosis. Eur. Neurol., In press.

Sibley, W.A. and J.M. Foley (1965): Infection and immunization in multiple sclerosis. Ann. N.Y. Acad. Sci. 122:457-468.

Printed in Sweden, Infotryck ab, Malmö 1981.197